- (3) M. A. Crenshaw, In *Skeletal Growth of Aquatic Organisms*, R. A. Lutz, Eds.; Plenum Press: New York, 1974.
- (4) A. Vies, B. Sabsay, In *Insights into Mineralization, Biomineralization, and Biological Metal Accumulation*, Westbroek, E. W. de Jong, Eds.; D. Reidel Pub., 1975.
- (5) S. Mann, J. Webb, R. J. P. Williams, Eds. *Biomineralization*, 1976; D. Reidel Pub., 1976.

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SYNTHETIC SURFACES AS MODELS FOR BIOMINERALIZATION SUBSTRATES

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ABSTRACT

Polyethylene and oxide substrates were derivatized with functional groups commonly associated with biomineralization substrates. These groups include carboxylate, phosphate, hydroxy, sulfonate, thiol, and amine. FTIR, XPS, and contact angle wetting were used to identify and characterize the products at each step. The efficacy of these groups toward inducing mineralization will be compared with naturally occurring substrates.

INTRODUCTION

Bone, dentin, and shell are a few of the examples of mineralized tissue found in nature (1). The structure and formation of these materials are of interest to materials scientists because these are composites of a mineral phase and a polymer phase (2-5). For example, shell, composed of approximately 95% mineral and 5% polymer, exhibits both high strength and fracture toughness compared to the individual constituents (6,7). The properties of mineralized tissue depend less on the materials used than upon the specific microstructure formed. Each microstructure is tailor-made to exhibit the desired properties.

These composite materials also represent a new and unique class of materials which has no conventional counterpart. Currently, no methods exist to produce a ceramic/polymer composite with a very high mineral content, nor can composites of any type be produced with the diversity and organization of microstructure observed in the lowly mollusk. Understanding the relationship between microstructure and properties will lead to improved composites of all types, not ceramic/polymer composites only. Understanding how these materials are made is critical to producing synthetic analogues.

The details of bone or shell formation have been dealt with in numerous reviews (2-5). However, in all systems an organic matrix or scaffolding is preformed which determines the geometrical characteristics of the microstructure. Upon this matrix are "nucleation proteins" or polysaccharides which provide sites that promote the formation of the mineral phase from a supersaturated solution. Cellular control of the mineralization step is limited to providing the requisite supersaturated solution; the actual nucleation and growth are determined by the organic nucleation site. While this view is certainly an oversimplification, producing synthetic surfaces that mimic the

biological nucleation matrix is the first important step in artificially mimicking the entire mineralization process.

The proteins or glycoproteins generally associated with mineralization are water soluble and contain a large fraction of acidic or polar amino acids. For example, the amino acids, aspartic acid, glutamic acid, and glutamine with the carboxylate and amide groups are frequently found in mollusk shell; hydroxy groups from serine and threonine are associated with silica deposition in diatoms; phosphate and sulfate groups attached to glycoproteins are commonly found in dentin and bone. Phosphate containing phospholipids are thought to be involved in the initial stages of bone formation. Interestingly, a variety of proteins and polysaccharides containing amine and thiol groups have NOT been associated with nucleation. These materials may simply not be available in the biological pathways associated with nucleation protein formation or they have some specific inhibiting effects towards nucleation. Either way, these are useful functional groups to compare with the set of groups listed above, which are important in mineralization.

Properties other than the type of functional group undoubtedly play an important role. A list of these would include: the matrix to which these groups are attached, the density of the groups, the mix of groups at a site, the ordering or structure of the groups relative to one another and to the growing nucleus, and the mobility of the groups. Recent work correlating the nucleation capacity with Langmuir-Blodgett film pressure succinctly demonstrates that group density and/or order is critical (5,8).

Our efforts have focused on determining the relative effectiveness of various types of functional groups with less emphasis on control of the surface's structure. Methods are described herein by which a series of functional groups can be attached to a common substrate using a common derivatization scheme. Ideally, this provides a system with various functional groups but an otherwise identical substrate with constant site density. The relative efficacy of the surfaces in promoting crystal growth will be compared in future work. Ultimately, these methods could be adapted to achieve control over site density and surface structure.

DERIVATIZATION OF POLYETHYLENE AND OXIDE SUBSTRATES.

The general scheme involves the attachment of a bifunctional straight chain alkane, $S-(CH_2)_n-R$, to the surface; where S is a functional group which covalently attaches to the substrate, and R is the desired functional group for mineralization. In most cases, however, R must be a protected group or a reactive intermediate which can be modified to the desired group after attachment of the alkane to the surface via the S group.

The inertness of polyethylene is both an advantage and a disadvantage. Because of the insolubility of polyethylene in most common organic solvents, it will not swell; therefore, derivatization can be confined to a shallow surface region. The problem resides in introducing a site to which the bifunctional alkane can be attached. Following the procedure developed by Rasmussen et al (9), shown in Scheme I, the polyethylene is first oxidized with chromic acid to introduce carboxylate groups.

Scheme I

Reaction with phosphorous pentachloride in ethyl ether converts the acid to the acyl chloride. A bifunctional alkane containing a primary amine, introduced in a basic solvent, such as triethylamine, reacts with the acyl chloride to form an amide linkage with the polymer. Specific procedures, which cannot be covered in detail here, depend on the solubility and reactivity of the alkane.

The bifunctional alkanes used were propylamine, N,N dimethylethylenediamine (DMED), β -alanine ethyl ester (β -Ala ester), and 3-aminopropanol. Propylamine, while containing the trivial methyl end group, provides a useful control substrate by which the influence of the amide linkage on mineralization can be determined. DMED provides a tertiary amine (tertiary amines are not reactive with acyl chlorides), while propanol amine provides a hydroxy group. As will be shown below, the hydroxy group is sufficiently unreactive with acyl chloride so little of the corresponding ester is formed. The protected β -Ala ester can be cleaved in 0.1 M HCl to give the β -alanine (β -Ala) derivative with the desired carboxylate group. Nominally, this surface is similar to the chromic acid etched surface but, as will be shown below, it appears to be more active than the etched surface.

The 3-aminopropanol derivative provides a simple route to the phosphate derivative. As in Scheme II, phosphorus oxychloride forms the alkoxyphosphochloride complex with the alcohol, which is further hydrolyzed to the phosphate in a weak sodium bicarbonate solution.

Scheme II

The bromopropylamine derivative is a useful intermediate for preparation of the corresponding thiol and sulfonic acid derivatives. Two routes, shown in Scheme III, are being evaluated for the preparation of the thiol. One involves preparation of the Bunte salt with sodium thiosulfate solution, which is converted to the thiol by treatment of acid. Alternatively, thiourea will displace the bromine to form an alkylisothiourea salt. Glycine hydrolyzes the isothiourea and treatment with acid gives the thiol. The sulfonic acid can be formed directly from the alkyl bromide using aqueous sodium sulfite followed by treatment with weak acid.

Scheme III

Preparation of the bromopropylamine derivative has posed some problems. While the hydrochloride salt of the β -Ala ester dissolved in pyridine is useful in preparing the amide derivative, the bromopropylamine hydrochloride reagent under similar conditions gives no reaction. Use of the free base should prove more satisfactory.

The procedure for derivatizing oxide surfaces makes use of alkylchlorosilanes or alkylalkoxysilanes to provide a covalent bond to the oxide surface. This method, illustrated in Scheme IV, can be achieved by deposition from the gas phase or from solution. To achieve monolayer coverage by deposition from the gas phase, scrupulously clean surfaces and careful control of water adsorbed to the oxide are critical. For this reason, we used a chamber which allows heating of the substrates up to 400°C and/or treatment with an oxygen RF plasma. The reader is referred to extensive literature on silane coupling agents for details (10).

Scheme IV

The procedures for attachment of specific functional groups are similar to that on polyethylene except that the choice of intermediates is more limited. The chlorosilanes are not compatible with amines, alcohols, and esters; the alkyl halide and alkyl nitriles are useful intermediates. We have focused on n-Bromopropyltrichlorosilane as the most promising species. Scheme V shows two successful methods for displacing the bromine with a carboxylate group.

Scheme V

The first process makes use of dilithoxyethylene which adds two additional carbons to the alkane. The second method uses methyl lithium to make the alkyl lithium derivative which is converted to the carboxylic acid with carbon dioxide. The bromoalkyl intermediate also provides routes to the sulfide and the sulfonic acid by methods analogous to those in Scheme III. Conversion of the alkylbromide to the alcohol will also provide a route to the phosphate derivative.

CHARACTERIZATION OF THE SUBSTRATES

One advantage of derivatizing solid substrates is the triviality of isolating the product; on the other hand, characterization of the surface requires sensitive surface analytical methods. Fourier transform infrared spectroscopy (FTIR) in the attenuated total reflection mode, X-ray photoelectron spectroscopy (XPS), and contact angle wetting (CAW) versus pH are the three convenient methods employed in this work. A variety of other methods, such as secondary ion mass spectrometry and solid state nuclear magnetic resonance, have also been used in similar analyses and will prove useful in future work.

A major difference between oxide and polyethylene substrates is the depth of derivatization. A true monolayer is formed on the oxide substrate, while derivatization of polyethylene has been shown to proceed to a depth of a few nanometers (9). For this reason, FTIR proves less sensitive for the oxide substrates than for the polyethylene substrates. Alternatively, XPS is highly sensitive on both substrates but is inferior to FTIR for qualitative analysis of the functional groups. For the sake of brevity the following discussion is limited to analysis of the polyethylene substrates.

Four intense sharp peaks appear at 2920 cm^{-1} , 2860 cm^{-1} , 1460 cm^{-1} , and 725 cm^{-1} in all spectra of derivatized polyethylene. Arising from vibrations of the polyethylene backbone, these peaks are of little diagnostic value. A small peak near 1710 cm^{-1} is due to the carbonyl stretch of aldehydes, ketones, carboxylic acids, esters, and amides. The position, shape, and behavior of this peak before and after derivatization are used to identify the product. Fig. 1 is the spectra of the $1400\text{--}1800\text{ cm}^{-1}$ region for unmodified polyethylene, carboxylated polyethylene, and the potassium salt of the carboxylated polyethylene. Unmodified polyethylene shows only fine

Fig. 1. FTIR spectra in the carbonyl stretch region of unmodified polyethylene (top), carboxylated polyethylene (middle), and the potassium salt of carboxylated polyethylene (bottom).

structure due to adventitiously adsorbed water; no carbonyl stretch is observed. A large carbonyl stretch at 1716 cm^{-1} is observed on carboxylated polyethylene. Upon conversion to the potassium salt, this peak shifts to 1574 cm^{-1} . A small peak remains at 1716 cm^{-1} which is due to aldehydes, ketones, or carboxylated groups still in acid form. Thus, the chromic acid etch is successful in introducing mostly carboxylate groups with some residual aldehydes, ketones, or buried carboxylate groups.

Fig. 2 shows the same $1400\text{--}1800\text{ cm}^{-1}$ regions for the polyethylene surface derivatized with propanol amine and DMED. A broad peak with a maximum near 1560 cm^{-1} is indicative of the carbonyl stretch of the amide. Also present is the 1716 cm^{-1} carbonyl peak attributed to unreacted carboxylate groups. This peak is smaller for 3-aminopropanol, reflecting an apparent greater degree of conversion to the amide compared with DMED. The reasons for this difference in reactivity are not clear and will be the objective of future work. If the hydroxy group of 3-aminopropanol had formed the respective ester rather than the amine, a shift of the carbonyl peak to higher wavenumbers would be expected. No such shift was observed, indicating that the amide is the dominant product.

Fig. 2. FTIR spectra in the carbonyl stretch region of the 3-aminopropanol and the N,N-dimethylethylenediamine derivatives on polyethylene.

Fig. 3 shows the FTIR spectra of the β -Ala ester derivative and the same surface after cleavage of the ester group. The relative intensity of the amide peak compared to the 1716 cm^{-1} peak should be reduced because of the presence of the additional ester or carboxylate groups. However, the intensity of the amide is reduced much more than might be expected. This is attributed to low reactivity of the β -Ala ester. The ester shows two peaks at 1716 cm^{-1} and 1736 cm^{-1} due to carboxylic acid groups on the polyethylene and the ester, respectively. Upon cleavage of the ester, the 1716 cm^{-1} peak is enhanced, indicating successful cleavage. The amide peak for the cleaved substrate is

reduced compared with the ester; this suggests some acid cleavage of the amide occurred.

Fig. 3. FTIR spectra of the carbonyl stretch region of the β -alanine ethyl ester derivative and the β -alanine free acid after cleavage of the ester.

Fig. 4 shows a series of XPS survey spectra for unmodified polyethylene, carboxylated polyethylene, the DMED derivative, the amino propanol derivative, and the phosphate derivative. The carbon (285 eV), oxygen (531 eV) and nitrogen (398 eV) peaks are of dominant importance. Unmodified polyethylene shows only a small oxygen peak due to atmospheric oxidation. Chromic acid etching significantly enhances the oxygen content. The absence of peaks due to chromium (575 eV) or sulfur (165 eV) indicates that chromic acid etching is not contaminating the substrates. The DMED derivative shows a combined amide and amine nitrogen peak as expected. As expected, the nitrogen peak is present for both the propanol and phosphatederivatives, indicating formation of the amide. The presence of the phosphorus 2s (194 eV) and 2p (137 eV) peaks strongly indicates formation of the phosphate group.

Fig. 5 shows contact angles versus pH for carboxylated polyethylene, the cleaved β -Ala derivative, and the DMED derivative. For carboxylated polyethylene, the trend with pH shows the expected decrease in wetting angle as the carboxylate groups are ionized. This is interpreted as additional confirmation of the presence of carboxylate groups. Carboxylated polyethylene does not wet strongly, with contact angles ranging from 83° to 77°. The inflection point of the curve is near pH 7 to pH 8, a value much higher than that expected for a typical alkylcarboxylate with a pK_a near 5. The result for the β -Ala derivative is similar. Stronger wetting occurs as indicated by the lower values of contact angle observed. The inflection point is shifted nearer pH 7. With the DMED derivative, the observed angles are lower than that for the carboxyl containing derivatives and show the expected increase in angle as pH is increased. This also confirms the presence of a basic functional group.

Fig. 4 XPS survey spectra of (from top to bottom) unmodified polyethylene, carboxylated polyethylene, the N,N-dimethylethylenediamine derivative, the 3-aminopropanol derivative and the corresponding phosphate derivative.

Fig. 5 Contact angle versus pH for carboxylated polyethylene (top), the cleaved β -alanine derivative (middle), and the N,N -dimethylethylenediamine derivative (bottom).

SUMMARY AND CONCLUSIONS

The relationship between the type of functional group attached to a substrate and its ability to promote nucleation and crystal growth have not been systematically explored. A series of surfaces which are identical, except for the specific functional group, would be required to do so. Here we present a series of schemes for attaching the important functional groups to polyethylene and to oxide substrates. FTIR, XPS, and contact angle wetting were used to characterize the products.

A nearly complete series of the targeted functional groups have been attached to polyethylene; while for oxide substrates the work is still in the early stages. However, the significant difference between the two systems lies not in the methods for attaching the functional group but in achieving attachment of an intermediate bifunctional alkane to the desired substrate. Once this is achieved, very similar methods can be used to convert the intermediate to the desired functional group. Thus, successful procedures on one substrate can be directly applied to another substrate.

Two important problems still need to be addressed. The reactivity of various bifunctional amines towards carboxylated polyethylene apparently varied considerably. This affects the relative density of groups attached to a surface. Systematic comparison of their ability to promote mineralization requires that this be constant. Related to this, but at this point less important, is the development of methods for absolutely quantifying the density of groups attached to a surface and development of methods for controlled variation of the density.

Ultimately, these surfaces must be used as substrates for mineralization experiments. Initial studies are in progress using calcium carbonate, lead iodide, calcium iodate, and iron oxide. These mineralization studies require a matrix of experimental conditions involving different methods of producing supersaturation, degrees of supersaturation, and temperature. Thus, the methods described above must be scaled up to produce tens or hundreds of substrates which are identical from batch to batch. This presents no fundamental problems, but does require a detailed understanding of the derivatization process and the influence of experimental parameters thereon.

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REFERENCES

- (1) H. A. Lowenstam, *Science*, **211**, 1126 (1981).
- (2) S. Weiner, *CRC Crit. Rev. Biochem.*, **20**, 365 (1986).

- (3) M. A. Crenshaw, In *Skeletal Growth of Aquatic Organisms*, D. C. Rhoads, R. A. Lutz, Eds.; Plenum Press: New York, 1980.
- (4) A. Vies, B. Sabsay, In *Insights into Mineralization, Biomineralization, and Biological Metal Accumulation*, P. Westbroek, E. W. de Jong, Eds.; D. Reidel Pub., 1983, pp 273.
- (5) S. Mann, J. Webb, R. J. P. Williams, Eds. *Biomineralization: Chemical and Biochemical Perspectives*, VCH Publishers, Deerfield Beach, Fl, 1989.
- (6) V. J. Laria, A. H. Heuer, this proceedings
- (7) M. Sarikaya, K. Gunnison, I. A. Aksay, this proceedings
- (8) B. R. Heywood, S. R. Rajam, J. D. Birchall, and S. Mann. *Biochem. Soc. Trans.*, 16, 824 (1988).
- (9) J. R. Rasmussen, D. E. Bergbreiter, G. M. Whitesides, *J. Am. Chem. Soc.*, 99, 4746 (1977).
- (10) E.P. Pleuddemann, *Silane Coupling Agents*, Plenum Press, New York, 1982.

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