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Biorelevant mesoporous silicon / polymer composites: directed assembly, disassembly, and controlled release

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Abstract We describe in this account a general, yet facile strategy for the directed assembly of bioactive composite materials comprised of an erodible organic polymer such as polycaprolactone and physiologically-resorbable inorganic mesoporous silicon. This method exploits a combination of capillary forces and selective interfacial coupling chemistry to produce isolable macroscale (mm sized) structures possessing a diverse range of geometries through simple mixing rather than intricate molding processes. Furthermore, we demonstrate the ability of such constructs to dissociate into their individual building blocks, with the concomitant release of embedded model compounds in a sustained manner.

Keywords Self assembly · Porous silicon · Polycaprolactone · Controlled release

1. Introduction

There exists a diverse number of strategies for the synthesis of nanoscale materials, some through the ubiquitous process of self-assembly (Whitesides, 1991). These patterning methods typically rely on a molecular template to achieve the desired order at the nanoscale, with species as diverse as surfactants (Kresge, 1992) and polynucleotides (Mirkin, 1996; Alivisatos, 1996; Coffer, 1996) capable of mediating such processes. In the continuing evolution of nanoscience and nanotechnology, a clear need exists, however, to link nanometer and micrometer-size objects into larger functional macroscale motifs, with control of structure & function a corresponding necessity – a point especially true for biomaterials applications.

Recent work by Whitesides and co-workers have clearly demonstrated the utility of capillary forces present at the interface of two immiscible interacting liquids (such as perfluorodecalin (PFD)/water) to produce self-assembled structures through mesoscopic assembly at a liquid-liquid interface (Terfort, 1997; Bowden, 1999; Bowden, 1997; Choi, 1999; Bowden, 2001; Gracias, 2002; Whitesides and Grzybowski, 2002; Ismagilov, 2002; Whitesides and Boncheva, 2002). Such studies are providing a unique perspective as to mechanistic details of diverse systems such as protein folding (Choi, 2000), nucleic acid hybridization (Weck, 2000), and crystal fracture (Thalladi, 2002). In these model systems, it is sufficient to exploit the relatively weak forces between assembled objects that arise from capillary interactions between hydrophobic-hydrophilic surfaces, albeit at a cost of non-isolable character in the self assembled structure. Nevertheless success in the formation of isolable mesoscale structures has been reported, however, with regard to helical electrical circuits based on a combination of capillary interactions (PFD/H_2O & solder/H_2O) (Gracias, Boncheva, et al., 2002). In our approach, the initial directionality is certainly facilitated by interactions between selective hydrophilic surfaces of biocompatible polycaprolactone (PCL) (Woodward, 1997) millimeter scale constructs (in this case cubes or hexagons) in a hydrophobic dispersion medium (a layered system of PFD and n-hexane), but our ability to produce isolated structures capable of ordinary handling entails

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the use of an erodible hydrophilic coupling reagent, such as a relatively inexpensive polysaccharide (starch), to achieve a reversible structure that is coupled at the monomer interface. By selecting a material such as mesoporous silicon in the composite building block formulation, the suitability of the overall three dimensional scaffold in applications such as orthopedic tissue engineering (Griffith, 2002) becomes apparent; this porous, spongy form of nanocrystalline Si has been shown previously to demonstrate bioactivity in simulated plasma whereby corrosion of the semiconductor with corresponding $Si(OH)_4$ release stimulates calcification along with a negligible inflammatory tissue response in vivo (Canham, 1995; Bowditch, 1999).

2. Experimental

2.1. Monomer building block fabrication

The structure with a particular shape was prepared first by making a polydimethylsiloxane (PDMS) mold of that particular shape (Bowden, 1999). For making hexagons of PCL, a hexagonal hollow rod PDMS was made by mixing polydimethyl siloxanes with a curing agent (5 wt% with respect to PDMS), wrapping the mixture around a hexagonal brass rod kept in a test tube, followed by heating at 110 °C for 30 min. After cooling, the brass rod was pulled out and breaking the test tube leads to the isolation of a hollow hexagonal PDMS mold. After obtaining the hollow, hexagonal PDMS mold, either only PCL (either in the form of small granules or small cut pieces) or a mixture of PCL and Si was poured inside the mold and heated at 110 °C for another 30 min. After cooling, the PDMS mold was peeled off to get the hexagonal rod of either PCL or PCL/Si composite material depending on the initial compositions of the starting materials used. The hexagonal rod was then cut with the help of a razor blade to obtain the individual hexagonal plates with the desired dimension.

2.2. Selective modification

Selective modification was carried out by embedding silicon powder at the selected sites of warmed PCL surface (faces or edges), below the melting pt.), followed by coating the Si-enriched sites with an aqueous solution of starch prior to the assembly process.

2.3. 2-dimensional assembly process

Typically, three 1, 3-modified cubes were placed in a 50 ml beaker (diameter 4.0 cm) containing 15.0 ml PFD and 10.0 ml n-hexane, rotating in an orbital shaker at a agitation rate of 200 rpm. To obtain linear chains of longer chain length, a larger vessel (800 ml beaker) containing 50 ml PFD and 50 ml n-hexanes rotating in the orbital shaker with an agitating rate of 90.0 rpm was employed. Once the assembly process is over, the liquid was removed and the assembled product was dried overnight in air at room temperature.

2.4. Calcification assays

All calcification assays were performed by soaking 3 mm samples (either Si-containing PCL cubes or PCL-only controls) in simulated body fluid (SBF), which consisted of salt concentrations very similar to that of human blood plasma (Canham, 1995; Kokubo, 1990). In the case of Si-containing cubes, the mesoporous Si (67%) was directly embedded along one face of the cube. The salt concentrations of the SBF consisted of 142.0 mM Na^+, 5.0 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 148.8 mM Cl^-, 4.2 mM HCO_3^-, 1.0 mM HPO_4^{2-}, and 0.5 mM SO_4^{2-}. The SBF solution was buffered at pH 7.40 with tris-hydroxymethylaminomethane and 1M HCl.

2.5. Cell culture assays

The above Si-containing PCL cubes were sterilized in 70% ethanol for 12 hrs. and irradiated with ultraviolet light. Transformed human kidney fibroblast cells (HEK 293) were obtained from American Type Culture Collection (ATCC Number: CRL-11268). Cells were cultured in 25-cm^2 tissue culture polystyrene flasks containing growth medium and maintained at 37°C in a humidified, 5% CO_2 atmosphere. The growth medium was composed of Dulbecco Modified Eagles Medium, 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, and an antibiotic cocktail consisting of 100 u/mL penicillin and 100 μg/mL streptomycin. For cell studies, cells were seeded at an initial density of 1×10^4 cells per 2 mL of medium and cultured in complete medium for 1 week, replacing the medium twice a week. Cell proliferation in the presence of the scaffolds was measured by counting cells at different time points using a Bright-Line Hemacytometer.

3. Results and discussion

3.1. Monomer building block fabrication

In the construction of the monomer building blocks comprised of Si and PCL, diverse opportunities abound. For a demonstration of the generality of the process we have initially exploited monomers of 2 different shapes, namely hexagons and cubes (Figure 1). Many of the experiments described herein entail cubes of 3 mm in length and hexagons with an opposite corner-to-corner distance of 3.75 mm and a corresponding thickness of 1.2 mm. These monomer blocks were initially fabricated by pouring molten PCL or PCL/Si mixtures into preformed silicone elastomeric molds and

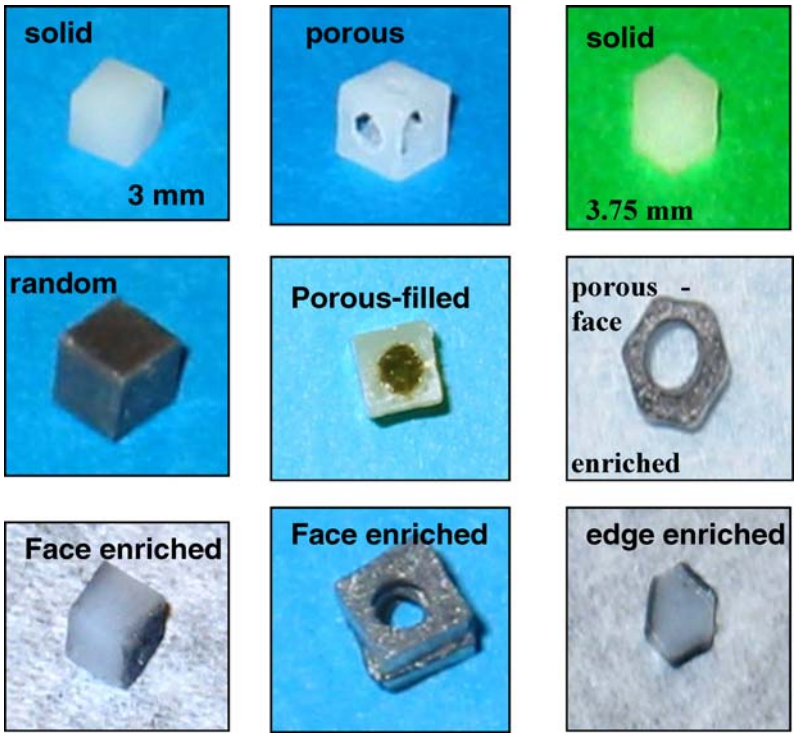

Fig. 1 Selected Si/PCL monomer composite building blocks

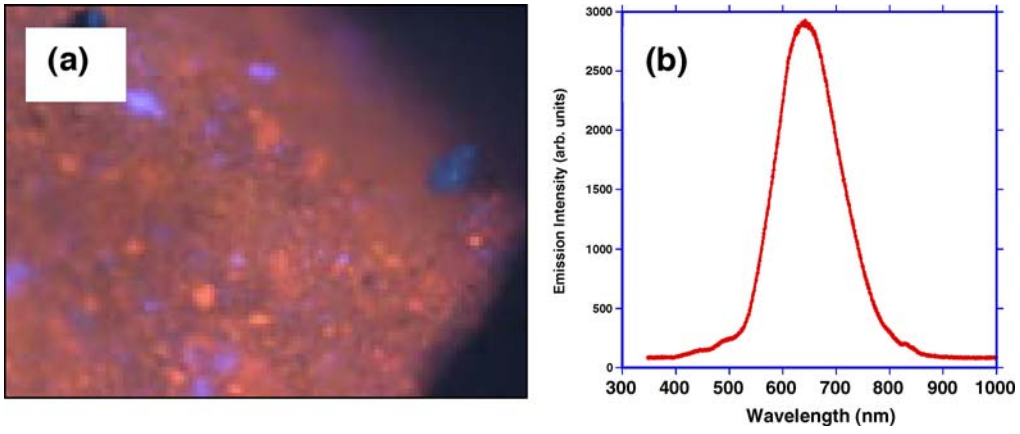

Fig. 2 (a) Fluorescent micrograph of a PCL cube containing luminescent porous Si (excitation wavelength of 370 nm); (b) corresponding photoluminescent spectrum of this cube

subsequently cutting each unit to its desired thickness. There are also numerous options for the choice of the Si material itself and its average location in a given building block. For the former, this includes amorphous, microcrystalline, or nanocrystalline Si. We have found mesoporous nanoscale Si of particular relevance in these materials, since it is known to be resorbable under physiological conditions and its elastic modulus is tunable as a function of porosity (Bellet, 1996). Furthermore, if desired, it is also possible to choose a form of porous Si that retains efficient photoluminescence in the visible region of the spectrum (Canham, 1990). As an illustration, a fluorescence micrograph and associated emission spectrum of a typical cube with orange-emitting porous Si embedded throughout is shown in Figure 2. Introduction of this type of Si into the composite is useful not only for spatial detection of the Si in the cube but has implications for possible diagnostic labeling as well. In addition to a uniform distribution throughout the polymer cube (or hexagon), the Si can also be preferentially enriched along selected face(s) or edge(s) of the structure. Deliberate porosity is introduced by physical indentation of 1, 2, or 3 orthogonal channels into a cube (Figure 1), and these interior locations also provide additional sites for selective Si localization. More subtle levels of porosity are also possible in the Si component itself by

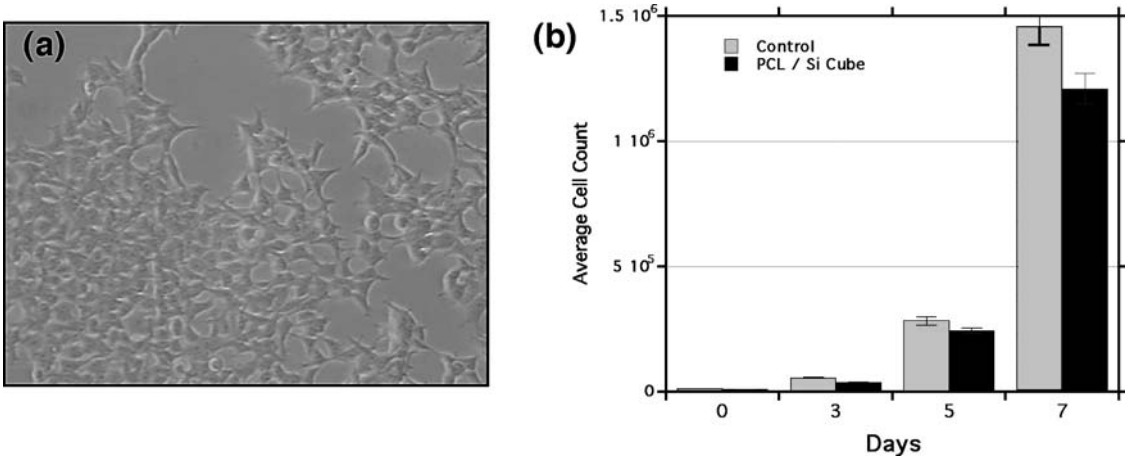

Fig. 3 (a) Optical micrograph of fibroblast cells in the presence of a PCL cube possessing mesoporous Si along one exposed face; (b) A plot of cell count versus time (days) for fibroblast cells in the presence of the above cube, as well as a control sample containing no Si or PCL

selecting either micro, meso, or macroporous Si as the relevant inorganic constituent material.

3.2. Biocompatibility/cytotoxicity assays

It is important to demonstrate that the individual building blocks do exhibit essential biorelevant properties. We begin by evaluating the toxicity of this type of composite by an analysis of the viability and proliferation of human kidney fibroblast cells (HEK 293) in the presence of a typical PCL cube containing mesoporous Si along one face; the Si in this case has a porosity of 67% and is added in the amount of 10 wt%. Fibroblasts are a prevalent cell line common to numerous connective tissue sites *in vivo*, and a useful starting point for *in vitro* screening of the utility of these materials. Both from a visual examination of cell density and morphology, along with a quantitative profile of cell count as a function of time, it is found that the presence of the Si-containing cubes results in a comparable proliferative behavior that is non-toxic to the cells (Figure 3). Such observations are consistent with the established behavior of both the mesoporous Si as well as PCL in terms of their biocompatible or bioactive behavior, as evaluated through *in vitro* and *in vivo* assays (Woodward, 1997; Canham, 1995; Bowditch, 1999).

To analyze their potential relevance to orthopedics, exposure of a given composite to a mixture of the cations and anions present in human plasma are a convenient assay; such solutions are typically referred to as 'Simulated Body Fluid' (SBF), as the serum proteins are absent in this medium (Kokubo, 1990). Results from a composite structure composed of 11.4% mesoporous Si along the interior of a one dimensional channel that has been exposed to a solution of SBF at 37°C for 14 days is illustrated in Figure 4. The SEM image clearly shows numerous calcified deposits, the composition of which can be detected in the corresponding energy dispersive x-ray spectrum. For a calcium phosphate

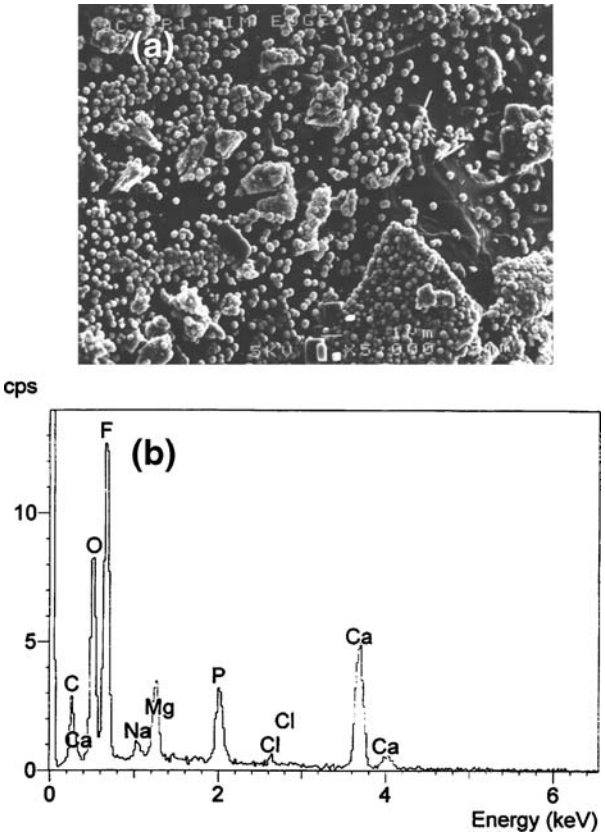

Fig. 4 Scanning electron micrograph of calcium phosphate deposits on mesoporous Si platelets which are embedded along the interior cylindrical surface of a porous PCL cube. (b) Energy dispersive x-ray spectrum illustrating the presence of calcium and phosphorous at such deposits

phase, the expected calcium and phosphorus signals near 3.9 and 2 keV, respectively, are clearly present. This result is in stark contrast with a control sample comprised solely of PCL, where there is an absence of calcified deposits on the surface of this material under the conditions utilized.

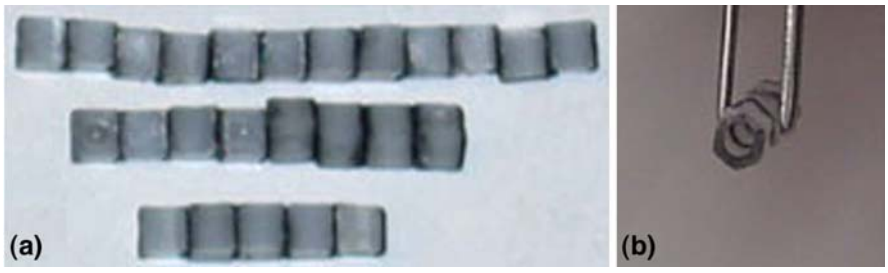

Fig. 5 (a) Linear assemblies of Si/PCL structures based on self-assembly of cubes selectively enriched in Si at opposite faces (1,3). (b) a similar structure constructed from mm-thick hexagons containing Si at adjacent faces

3.3. Self-assembly experiments

For the self-assembly experiments, it is most convenient to classify the range of possible structures as a function of dimension. It is possible to form one dimensional (1-D) stacks by mixing monomers at a PFD/hexane interface, producing metastable structures stabilized solely by hydrophobic/hydrophobic interactions. However, isolable one dimensional chains are most readily formed by selective enrichment of silicon on opposite faces of a given cube (referred to herein as a 1,3 modified structure) or hexagon (a 1,4 structure), followed by treatment with a relatively concentrated starch solution and mixing at a PFD/hexane interface for 10 minutes (Figure 5a). The initial attraction between cubes can be understood in part in terms of hydrophilic attractions between oxide terminated Si surfaces, the geometric orientation of which is affected by the relative density of our composite objects to remain buoyant through menisci at the solvent system employed (Bowden, 2001). This is certainly compatible with the selection of PFD (d = 1.917 g/mL) and hexane (0.660 g/mL) layers mediating the assembly of PCL cubes possessing a density of 1.145 g/mL. Such conditions produce negative menisci for the hydrophilic Si-containing faces. The resultant capillary forces are known to be sufficient to form ordered arrays under relatively mild mixing rates ($\sim$0.75 sec^{-1}).

As a part of ensuring a strong interaction between the coupling agent and the Si surface, it is useful to heat the crystalline Si alone in an oxidizing environment at slightly elevated temperatures (such as 80°C) prior to cube fabrication in order to ensure that an adequate amount of oxide is present at embedded Si surfaces. In terms of specific chemistry between the Si and the starch, it is the hydrogen bonding interactions which presumably drive the observed coupling and permits the formation of relatively long isolable structures. The ultimate length of such structures are limited by the width of the mixing vessel, but we have readily formed linear chains of solid cubes up to 3.6 cm in length. In each case, a remarkable selectivity of coupling only between the modified Si faces is observed. It should also be noted that these assembly processes are not limited only to solid cubes or hexagons, but readily adapted to monomer building blocks possessing one, two, or three porous cylinders whereby the overall structure formed possesses an aligned channel throughout (Figure 5b). In such cases, additional diversity is available for such porous cubes by incorporating bioactive nanoscale silicon into the interior channel, if desired. It should also be stressed that for control coupling experiments containing mixtures of Si-modified & unmodified cubes in the presence of starch, only modified cubes were observed to couple with each other; unmodified PCL cubes did not participate in coupling at all.

Compositional characterization of these assembled interfaces is achieved using micro-Raman spectroscopy. Beginning at a PCL-rich region of a dimer structure coupled along Si-rich faces using starch, only vibrational modes associated with the polymer are observed at 1100, 1300, 1440, and 1730 $cm.^{-1}$ Moving toward the interface, the relatively strong peak associated with the crystalline Si phonon is detected near 520 cm^{-1}, along with a broad vibration near 950 cm^{-1} attributed to the starch coupling reagent (Figure 6). Emerging at the boundary between these two regions is a hybrid convolution of the peaks belonging to all three components.

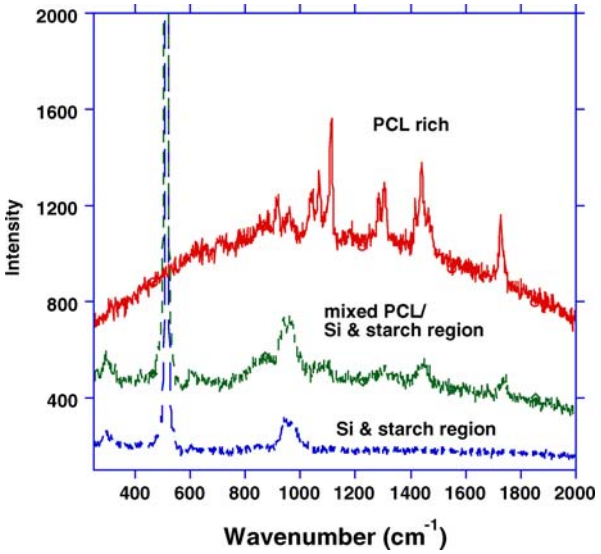

Fig. 6 Micro-Raman spectra illustrating the different spectral signatures associated with PCL-rich (top spectrum), Si & starch–rich (bottom spectrum), and interfacial boundary regions (center spectrum) of an assembled dimer structure

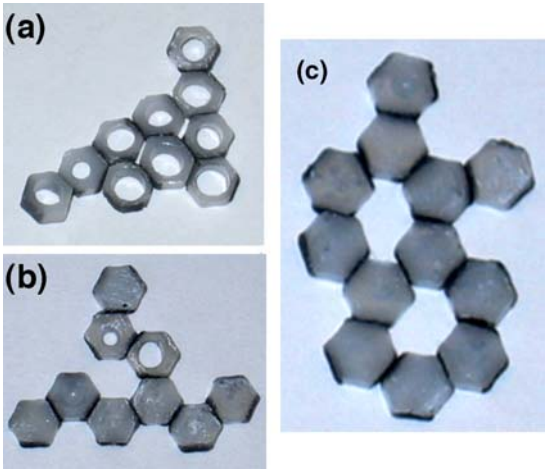

Fig. 7 Optical micrographs illustrating assemblies formed from the use of selective three face Si enrichment of PCL hexagons. (a) Condensed structures formed from building blocks with Si at edges 1,2, and 4; (b),(c) Open and branched structures formed from monomers with Si at edges 1,3, and 5

From a geometric perspective, more complex two-dimensional structures are expected if a second or third face or edge is embedded with Si. To illustrate some of the more complex two-dimensional patterns possible with hexagons possessing three edges modified with Si, we have carried out preliminary experiments involving the assembly of these monomers with the 1,2,4 or 1,3,5 edges Si enriched. When porous hexagons modified in the 1, 2 and 4 positions are allowed to couple in the starch medium, they produce closest packed 2-D 'honeycomb'-like structures (Figure 7a). In this case, once a critical surface area for starch-mediated Si coupling is reached, condensed structures are formed which may possess an occasional mismatch (i.e. loss of selectively at one face in the condensed structure). Interestingly, however, separation of the Si-enriched faces in terms of a 1,3,5 edge-enriched monomer makes branched, open structures possible (Figure 7b).

3.4. Dimer disassembly and reagent release

It is crucial to note that these starch-mediated interactions are reversible and can be hydrolyzed into the monomer building blocks. The average kinetics of the disassembly process depends on several factors: starch concentration, post-assembly drying time, and rate of stirring, if any. Most reproducible results are obtained with the use of a 2% starch solution (w/w), a 2.5 hr drying time, and controlled agitation at 50 rpm in a shaker platform. By using the point at which physical separation of the cubes can be discerned with the unaided eye as a working definition of dissociation time, an average value of 23 minutes is found.

As a model system, we analyzed the release of the widely-studied transition metal complex $Ru(bpy)_3Cl_2$, a species previously investigated in solar energy-related research (Bard, 1995), polynucleotide binding (Erkkila, 1999), and controlled release from porous Si films (Li, 1998; Li, 2002; Li, 2003). Variants of the monomer building blocks were constructed whereby a single mm–wide hole approximately 1 mm deep was formed into a single face of a given cube; requisite amounts of stock solution of $Ru(bpy)_3Cl_2$ are added to the orifice and allowed to dry. The Si is then added, and the structure assembled with starch. The second variant of these dye-embedded cubes entails first the addition of Si to the cube face, formation of a recessed 1 mm deep hole along this modified face, then adding the dye. After drying, the structure is assembled in the usual manner. In these experiments, a range of dye concentrations varying from 3.2 to 85 μg was loaded into the cubes.

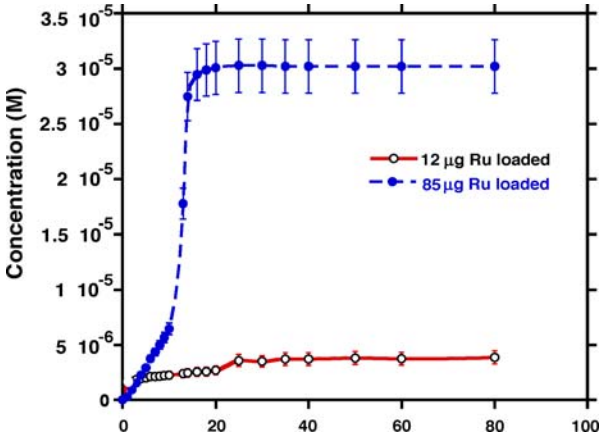

Fig. 8 Typical release profiles for Si/PCL dimer assemblies loaded with (a) 85 μg and (b) 12 μg of $Ru(bpy)_3Cl_2$upon immersion in water

Typical diffusion profiles for dimer assemblies loaded with (a) 85 μg and (b) 12 μg of $Ru(bpy)_3Cl_2$are shown in Figure 8. Note the inflection point observed in the time interval when physical dissociation of the dimer is typically discerned, at approximately 20 minutes. In general it is found that the amount of dye delivered to the surroundings is proportional to the original amount loaded into a given cube, as expected, with the amounts delivered ranging from 2.8 μg to 58 μg. The average percent dye released to the surroundings, based on a single immersion event in water, is approximately 70 percent. Furthermore, the ability of the Si to act as a diffusion barrier is a relative function of the amount of dye loaded initially; for example, if the dye is loaded at 12 μg onto the recessed 'bowl' in a cube and then covered with Si, the initial rate of diffusion (0.145 min^{-1} ; i.e. the slope prior to the inflection point) is five times slower than that for the assembly when the Si is added first to the cube, followed by the dye (0.027 min^{-1}). This is presumably a consequence of a larger burst effect of dye release taking place when excess dye is present on the surface of the cube. If a lower concentration of dye is added, then the initial diffusion rates are statistically

comparable. Hence the properties of the disassembled structures are also tunable by the relative hierarchy in which they are constructed. This result is of relevance in the design of additional structures possessing the proper dimensions that would release therapeutically useful substances from mesoporous Si. It suggests that release kinetics can be modulated by the use of silicon acting as a porous gate screening the delivery through simple architectural control.

4. Conclusions

We believe that the approach described above provides an extremely general route to the controlled formation of a wide range of useful biologically relevant composites possessing selectively formed architectures. The diversity of materials accessible by such methods are property-tunable not only as a consequence of spatial organization of the inorganic component of the composite, but also in the feature size of the Si (for example) and the desired formulation of the resorbable polymeric host. Further explorations in these and additional areas are in progress.

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