Biofilm origin of clay-coated sand grains

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ABSTRACT
The presence of clay-sized particles and clay minerals in modern sands and ancient sandstones has long presented an interesting problem, because primary depositional processes tend to lead to physical separation of fine- and coarse-grained materials. Numerous processes have been invoked to explain the common presence of clay minerals in sandstones, including infiltration, the codeposition of flocculated muds, and bioturbation-induced sediment mixing. How and why clay minerals form as grain coats at the site of deposition remains uncertain, despite clay-coated sand grains being of paramount importance for subsequent diagenetic sandstone properties. We have identified a new biofilm mechanism that explains clay material attachment to sand grain surfaces that leads to the production of detrital clay coats. This study focuses on a modern estuary using a combination of field work, scanning electron microscopy, petrography, biomarker analysis, and Raman spectroscopy to provide evidence of the pivotal role that biofilms play in the formation of clay-coated sand grains. This study shows that within modern marginal marine systems, clay coats primarily result from adhesive biofilms. This bio-mineral interaction potentially revolutionizes the understanding of clay-coated sand grains and offers a first step to enhanced reservoir quality prediction in ancient and deeply buried sandstones.

INTRODUCTION
Bio-sediment interactions (microbially induced sedimentary structures) have been recognized in rocks from almost all geological time periods (Noffke et al., 2006). Biofilms have been shown to play a fundamental role in sediment dynamics and the subsequent diagenesis of marginal marine sedimentary systems (Stal, 2003), affecting grain-size heterogeneity (Garwood et al., 2015), sediment stability (Vignaga et al., 2013), sediment transport, and bedform stability (Malarkey et al., 2015; Schindler et al., 2015). Intertidal biofilms typically result from the secretion of extracellular polymeric substances (EPS) (adhesive mucilage) by microphytobenthic (MPB) communities that are composed of algae (diatoms, Euglenids, cryptophyceans, dinoflagellates), cyanobacteria, and other photosynthetic bacteria (Jesus et al., 2009). This study focused primarily on the role of silicate phototrophic epipelic (motile) diatoms, which represent the dominant microorganism within intertidal sediments of western Europe (Stal, 2003; Underwood and Paterson, 1993).

EPS (colloquially known as bio-glue and mucilage; Agogué et al., 2014; Higgins et al., 2003) is produced within intertidal siliciclastic estuarine settings by epipelitic diatoms for a variety of functions (Decho, 1990). One function of EPS is to facilitate the vertical movement of diatoms, which colonize the sediment in response to tidal and daylight cycles, for photosynthetic purposes and to maintain optimum environmental conditions (Hoagland et al., 1993; Stal, 2003). Excreted EPS strands allow diatom movement in near-surface sediment, but they also anchor diatoms to sand grain surfaces. The strands detach from the diatom on movement and remain on mineral grain surfaces; this produces a web of bridging and coating mucus (biofilm) strands on grain mineral surfaces (Garwood et al., 2015; Higgins et al., 2003).

Biofilms in intertidal sediments have been reported to form an adhesive coat on sand grains that acts as a binding agent capable of forming aggregates of diatoms, organics, and clay minerals (Kessarkar et al., 2010) and that is also responsible for fine particle entrainment in sand-dominated estuarine tidal flats (Garwood et al., 2015). In the presence of common divalent cations, such as Mg$^{2+}$ and Ca$^{2+}$, that are present in estuarine waters, EPS biofilm fractions become water insoluble, tightly bound to sand grain surfaces, and resistant to degradation, increasing with depth in the top few millimeters of the sediment column (Stal, 2003; De Winder et al., 1999).

The goal of this study was to establish the mechanism by which clay-grade particles overcome hydrodynamic segregation and are physically bound in a sand-rich deposit. Specifically we sought to determine how clay minerals adhere to sand grain surfaces. Our main hypotheses were that biofilms control the formation of clay grain coats, and that the amount of biofilm present directly affects the degree to which sand grains are coated with clay minerals.

DATA SETS AND METHODS
Surface sediment samples were collected from the Ravenglass Estuary in northwest England (Bousher, 1999). This estuary is a modern analogue that is equivalent to the environment of deposition for an estimated 54% of all chloride clay-coated sandstones (Dowey et al., 2012).

Textural and chemical classification included a range of scanning electron microscopy (SEM), environmental SEM, and Raman spectroscopy on a suite of 112 polished thin sections; 112 dried, loose sediment grain mounts; and 112 natural, undried (hydrated) sediment samples. The samples were analyzed for biofilm abundance using the established biomarker proxy chlorophyll-a (Stal, 2003; Underwood and Paterson, 1993), as well as clay coat textural characterization, clay coat chemical analysis, and percentage of clay coat grain coverage.

Grain clay-coverage was quantified using polished thin sections of grain mounts. The method used involved point counting 50 sand grains per sample and measuring the total perimeter of each sand grain and the length that is covered by attached clay coats using Petrog statistical software (Pantopoulous and Zelilidis, 2012). Repeat analyses of the same sample indicated an average ±1.7% error for mean clay-coat coverage quantification.

We collected 97 surface sediment samples on 19 and 20 May 2016, within 4 hours of low tide. The material was collected within sterilized foil, stored on ice during collection, placed in a freezer at −18 °C for transport, and then at −80 °C within 4 hours. Standards and samples were handled in subdued light during analysis. Chlorophyll-a samples and calibration standards were prepared in 90% acetone. Sediment samples (200 mg) were weighed and 2 mL of 90% acetone added and mixed. Method blanks (no sediment) were treated in the same manner as the samples. The extracts were transferred and filtered through a 0.4 µm Phenex RC membrane. A Trilogy fluorometer (model 7200–000) measured the fluorescence of the 90% acetone, blanks, standards, and samples. A standard curve was generated to calculate chlorophyll-a concentration using the formula chlorophyll-a concentration (µg/g) = [fluorescence units/slope] × [dilution (µL)/weight extracted (g)].

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TEXTURAL AND CHEMICAL CHARACTERIZATION OF CLAY-COATED SAND GRAINS

Clay-coated sand grains were observed to contain a network of fine fibrous filaments that link sand grains in the modern Ravenglass marginal-marine environment (Fig. 1B). The filaments produce a framework that binds together finer grained detrital material, such as clay minerals, organics, and silt-grade lithic clasts. This thin film of extraneous material was found to partially coat sand grains, leaving other parts of sand grains with clean, uncoated surfaces (Fig. 1C). The film appears to have adhesive properties, because fine-grained clay- to silt-size materials are stuck on the part of the grain covered with the film but not on the film-free parts of sand grains. Thus the film appears to act as an adhesive, creating aggregates composed of clay- to silt-sized detritus stuck to sand grain surfaces. It is also noteworthy that diatoms can be observed anchored to sand grains in the vicinity of adhesive films (Fig. 1C). In hydrated (natural) form, clay coats are discontinuous, with tangentially orientated clay flakes on the parts of sand grain surfaces that are covered with the adhesive film, with no observed supporting clay matrix. Clay in the sediment is exclusively observed as discontinuous clay coatings or bridging structures (Figs. 1A–1C).

Raman spectroscopy was employed to generate a spatially resolved chemical signature of the film, specifically to determine whether the film on the sand-grain surfaces was organic. The Raman spectrum (Fig. 2), generated from a film on a representative clay-coated sand grain, contains molecular bands that are fully consistent with organic-rich complex mixtures that are typical of EPS mucilage and biofilm-specific polysaccharides (Ivleva et al., 2009). Diatoms are directly anchored to sand-grain surfaces by excreted EPS (Fig. 1C).

DISTRIBUTION OF SEDIMENT CLAY-COATED GRAINS AND BIOFILM ABUNDANCE

The degree of sand grain clay-coat coverage ranges from 0.5% to 87% within sediment at the surface of the Ravenglass Estuary (Fig. 3A). The distribution of clay-coat coverage has been mapped for the estuary using interpolation in ArcGIS (https://www.arcgis.com). This revealed a highly heterogeneous pattern (Fig. 3A). Clay-coat coverage in the estuarine sediment increases in a landward direction, and is most extensive within the mid- to upper estuary, in deposits representing tidal flat and tidal bar depositional environments (Wooldridge et al., 2017).

Chlorophyll-a concentration in the estuarine sediment varies from 0.4 µg/g to 45 µg/g. Chlorophyll-a concentration has been shown to be a proxy for diatom-produced biofilm abundance within sediments (Stal, 2003), indicating that biofilms are not homogeneously distributed across the estuary. We have used ArcGIS to map the heterogeneous distribution of chlorophyll-a within the estuary, thus revealing the heterogeneous distribution of biofilms by proxy (Fig. 3B). Chlorophyll-a increases in concentration in a landward direction, and is most extensive within the mid- to upper estuarine tidal flats. The chlorophyll-a distribution is supported by other, significantly less detailed, studies of tidal flats (Stal, 2003; Underwood and Paterson, 1993).

A Pearson’s correlation coefficient was used to test the statistical significance between chlorophyll-a (biofilm) abundance and clay-coat coverage. This approach revealed a Pearson’s correlation of 0.745 with a P value of <0.001, confirming a very strong statistical link between the degree of clay-coat coverage and sediment biofilm abundance (Fig. 4).

ORIGIN OF CLAY MINERAL ATTACHMENT

The textural characteristics of the sediments are consistent with biofilms produced by diatoms (Higgins et al., 2003), as reported in studies of both modern (natural) estuaries (Kessarkar et al., 2010; Tolhurst et al., 2003) and laboratory-based experiments (Malarkey et al., 2015; Paterson, 1989). Our study found textures that are consistent with reported diatom trails and linkages (Fig. 1), upon and between grains, that form due to the movement-related production of EPS secretions (Higgins et al., 2003).

Figure 1. Backscattered electron and environmental scanning electron microscope (SEM) images of clay-coated grains. Arrows indicate clay coatings. Dashed lines outline the extent of the biofilm coats on the grain surface. A: SEM image of loose sediment. B: Thin section of clay-coated sand grains from an intertidal estuarine setting. C: Environmental SEM image of hydrated sediment. Triangle in top right points to a diatom with excreted extracellular polymeric substance grain attachment outlined.

Figure 2. Laser Raman fingerprint of a clay-coated grain. The Raman spectrum is of the tidal flat sediment, imaged in Figure 1. The chemical signature is representative of an organic-rich mixture that is typical of a biofilm (Ivleva et al., 2009). Abbreviations: asymm—asymmetrical; str—stretching.

Figure 3. Maps of clay-coated sand grain distribution and biomarker proxy for tidal flat biofilm abundance on sand grains. A: Clay-coat coverage of sand grains established from quantitative petrography. Rivers Irt, Mite, and Esk are indicated. B: Chlorophyll-a concentrations. Darker shades in A and B represent greater extent of clay-coat coverage and greater abundance of sediment biofilm (chl-a—chlorophyll-a).

Figure 4. Cross plot of percentage clay-coat coverage and biofilm biomarker (chl-a—chlorophyll-a) indicating a positive correlation between increased sediment biofilm abundance and clay-coat coverage.
The adhesive properties of biofilms (Higgins et al., 2003) are apparent because they attach clay material to sand-grain surfaces, and can form a linked sediment-clay string between grains (Figs. 1C and 1D). Raman spectroscopy further confirms that the texturally identified films have a biological origin and can be correctly identified as biofilms (Fig. 4).

Chlorophyll-a abundance is controlled by the environmental niche of MPB communities. Diatoms of this type become stressed in sediments that undergo turbulent conditions (such as those typically found in lower estuary locations), and therefore they become less abundant (Stal, 2003). They also become stressed at sites that have relatively short periods of exposure to light, for example, in outer tidal flats where there is limited exposure to full daylight at low tide (Stal, 2003; Underwood and Paterson, 1993).

The identified heterogeneous distribution of diatoms (environmental niche) and their EPS secretions result in some grains being coated with adhesive EPS and others remaining uncoated, thus resulting in different degrees of clay material adhesion to grain surfaces across an estuarine system (Fig. 3). The spatial distributions of clay-coat abundance and chlorophyll-a are remarkably similar (Figs. 3A and 3B).

We thus propose that the origin and distribution of clay-coated sand grains are primarily controlled by biofilms, based on (1) the similarities of the textures of the clay coats in our samples to those reported to be due to biofilms (Fig. 1) (Malarkey et al., 2015; Vos et al., 1988), (2) the compositional identification of organic biofilm compounds within the adhesive film on which clay material is attached (Fig. 2), and (3) the close correspondence of the distribution of clay-coat coverage and sediment biofilm abundance (chlorophyll-a) (Figs. 3A and 3B) and the positive statistical correlation (Fig. 4).

This newly proposed biofilm origin of clay-coated sand grains, involving a central role for marginal marine, epipelic diatoms, does not entirely contradict previously reported explanations for clay coats. Instead, our work has revealed a bio-glue mechanism of clay particle attachment during which clay material, introduced via bioturbation by macrofaunal organisms (Worden et al., 2006), codeposition, or infiltration (Wooldridge et al., 2017; Wilson, 1992), could be attached to sand-grain surfaces. Our proposal is that diatoms (MPB) produce a biofilm that acts as a bio-glue that coats and bridges sand grains, producing a sticky web (EPS) that binds and traps codeposited and later-infiltrated clay particles.

There is a reduction in both biofilm abundance (Fig. 3B) and clay-coat coverage from the inner to outer estuary (Fig. 3A). Note that clay coats are found, albeit at low abundance (5%–10% coat coverage), at sites where biofilms are at their lowest concentration (1–5 μg/g; e.g., in parts of the main channel). This may represent transportation of clay-coated grains from the site of greatest biofilm concentration in the inner estuary (i.e., the clay coat factory) out of the estuary via the main channel, accompanied by a degree of abrasive clay-coat degradation due to grain collisions (Wilson, 1992). As a consequence, with transport, inner estuary biofilm-mediated clay coatings persist as partial attached coats, potentially explaining the weaker spatial correlation in the main channel (Fig. 3).

**IMPLICATIONS FOR CLAY-COATED SAND GRAINS IN SANDSTONES**

The clay-coated sand grains reported in this study potentially represent precursor coats to those found in numerous deeply buried sandstones (Dowey et al., 2012). Clay-coated grains in sandstones are of interest because they affect petroleum reservoir quality; they inhibit quartz cement growth during prolonged burial and heating (Aagaard et al., 2000; Bloch et al., 2002; Worden and Morad, 2000). If, as reported by Bloch et al. (2002), burial diagenetic clay coats result from recrystallization of clay coats formed during deposition, then developing a robust understanding of the origin of the primary coats will lead to models capable of predicting reservoir quality in sandstones (Wooldridge et al., 2017). It should thus be noted that diatom (MPB) biofilm-producing organisms have been reported previously from other modern estuarine environments (Paterson et al., 2000) and deltaic sediments (Delgado, 1989). This has implications for a possible consistent biofilm-mediated origin for the reported similar clay-coated sand grain textures within such marginal marine sedimentary systems (Dowey et al., 2017).

Clay-coated sand grains are not limited to marginal marine sediments, but the link between biofilms and coats remains unconstrained. However, biofilms have been reported to attach dust particles to sand-grain surfaces in desert environments (Belnap and Weber, 2013). Clay coats have been reported in meander fluvial point-bar environments (Wooldridge et al., 2017), in which biofilm abundance is comparable to that in estuarine sediments (Gerbersdorf et al., 2008).

Biofilm (EPS) bound aggregates of clay particles and freshwater diatoms on sand grains have been reported in marine sediment 27 km from the nearest river mouth, implying that biofilm attachments remain effective during transport from the inner estuary clay coat factory into the marine realm (Kessarkar et al., 2010). A marginal marine, primary origin of clay-coated grains has been suggested within the continental slope (sediment gravity flows) and basin plain sediments of the Atoka Formation, Arkoma basin, central USA (Bloch et al., 2002; Houseknecht and Ross, 1992) and west of Shetland, UK (Sullivan et al., 1999). These reports suggest that biofilm-bound clay-coated sand grains that develop within estuarine setting may be resistant to sediment reworking and transport. The biofilm origin of clay-coated sand grains reported here may also explain the origin of clay-coated grains within continental shelf and slope, and basin plain sediments (Dowey et al., 2012).

**CONCLUSION**

This paper documents a novel biological mechanism that explains the origin of clay-coated sand grains in modern marginal marine sediments. We show that clay particle attachment is mediated by a biofilm. The discovery of a biological-biofilm mechanism of clay-coat formation in sediment at or near the Earth’s surface is of great significance and achieves a crucial step in understanding and predicting the distribution of clay coats in deeply buried sandstone reservoirs.

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