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On Diatom Colonization of Porous UHMWPE Scaffolds

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***Abstract*— Diatom species *Karayevia amoena* and three species of *Halamphora coffeaeformis* were used to colonize open-cell porous UHMWPE scaffolds with ca. 70 vol.% porosity during 20-days laboratory exposure to the media drawn from the Baltic Sea (Baltiysk, Russia) and Arabian sea (Mumbai, India), respectively. The nature of diatomic colonization of various surface relief elements were studied by means of SEM imaging of dried specimens. Individual diatoms, their aggregates and extended colonies occupy 'valley' and 'hill' elements of relief. forming single and multi-layer structures. *Karayevia amoena* diatoms strongly attach themselves to the substrate, with the evidence of polymer substrate deformation seen in the vicinity of diatom exoskeleton. The mechanisms of diatom proliferation and the formation of intriguing 3D "pot-shaped" colonies are examined in view of the force interaction at the diatom-substrate interface.**

***Index Terms*— Diatoms, UHMWPE, sustainable materials, aquaculture**

I. Introduction

DIATOM algae play an important role in hydrosphere ecology due to the combination of fascinating structure and biomechanical performance. Diatoms attract attention from various research communities, including marine biologists and ecologists, and more recently from materials scientists and engineers [1]. Many important aspects of their physiology and interaction with different substrates have been intensively studied [2], but many questions remain open. Deeper understanding must be advanced through the use of various characterization methods, including most advanced techniques, such as FIB-SEM imaging [3]. The objective of such studies is to expand the range of applications of diatoms, as they can be used as a sustainable resource for the production of biofuels, biominerals, and bio-materials engineering [4]. Another potential application of diatoms is for the bioremediation of polymers that pollute the hydrosphere [5].

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Ultra high molecular weight polyethylene (UHMWPE) is a high mechanical performance polymer that finds application in naval technology, e.g. for ropes and sails, since its commercialization in 1990s [6]. Due to high bioinertness, acceptable mechanical properties and wear performance UHMWPE finds growing usage in surgery as bone and joints implants, and more recently in cellular structures as scaffolds for tissue engineering [7]. When colonized with mesenchymal stromal cells (MSC's), open-cell porous UHMWPE scaffolds show high capacity for osseointegration and vascularization [8].

The underlying idea of the present research study was to examine the diatom algae colonization of UHMWPE porous scaffolds, with a view to create a new class of sustainable materials that could be called diatom/polymer hybrids (DPH). A number of modes and processes accompanying the colonization of cellular polymer can be envisaged:

a. "Superficial colonization" that is characterized by the absence of algae progression into the sample depth
b. "Deposition colonization", in which a thick diatomite-ceramic porous layer is grown which may have interesting barrier or filter properties.
c. "Digestive colonization" is characterized by the consumption of the substrate polymer by diatom activity which leads to its progressive conversion due to bio-degradation. Progression of this process would then lead to complete decomposition of polymer articles, and can be used as a basis for pollution remediation technology.
d. "Volume colonization" is associated with intensive proliferation of diatoms into the depth of the polymer scaffold. This scenario may create a sustainable bulk diatom/polymer hybrid with potential engineering applications exploiting the structural, tribological, thermal, vibrational, and acoustic properties of the composite. UHMWPE is well known to be chemically stable and biologically inert, and is an ideal candidate for superficial and volume colonization. In this report we present the initial results of investigating the structural aspects of the interaction between diatom algae colonies and porous UHMWPE scaffold substrates.

II. Materials and Methods

The samples of UHMWPE sponge were produced in accordance with the method presented previously [9]. The mixture of UHMWPE powder (molecular weight of 5×10^6 g/mol, 4120 GUR Ticona®) and food grade rock salt (NaCl)

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with the powder particle size ranging from 80 to 700 µm. The dry mixture with the component weight ratio 1:9 was gently stirred using Fritsch Pulverisette 5 (Fritsch GmbH, Germany) planetary ball mill in agate vessels (500 ml) filled with 8 mm diameter corundum grinding balls. Thermal pressing was carried out under the load of 70 MPa at 180 ºC. Salt removal was carried out using distilled water at 60 ºC by means of ultrasonically assisted washing as the final stage of substrate manufacturing. This resulted in the formation of open cell porous structure with about 80% volume porosity.

Sea microalgae cultures for the colonization of UHMWPE substrates were collected from two micro-phytobenthic probes taken from littoral soils. These were cultivated at ambient temperature in diffuse daylight for the duration of one year using the polyester substrate of micro-aquarium walls, ultimately forming areas of dense diatom coverage. The first population was cultivated from the probe collected at Kaliningrad gulf (Baltic Sea, Russia) littoral at a salinity of 5 ppt and was represented by *Karayevia amoena* algae (98,5%). The second population was cultivated from the probe collected at Mumbai (Arabian Sea, India) littoral at the salinity of 30 ppt, represented by *Halamphora* genus diatoms (93,4%).

In order to promote the colonization, samples of porous UHMWPE were suspended in the water column and placed in a laboratory micro-aquarium in the proximity (5-10 mm) of dense diatom coverage for 21 days at ambient temperature, with intense light emitted by the LED1106 G2 2.3W, 18 mA, 35 lm/W lamp placed 450 mm from the micro-aquarium wall, with the angle of light incidence of 30-40˚. Three samples of porous UHMWPE were colonized with each diatom culture to ensure the statistical robustness of the results obtained.

SEM and FIB-SEM imaging of colonized scaffolds were performed using low-vacuum scanning electron microscope Hitachi TM-1000 using BSE detector. Further details of diatom-substrate interactions were obtained using Tescan MAIA3 SEM and Tescan LYRA3 FIB-SEM microscopes. The specimens for microscopy sessions were washed in distilled water and dried in three sequential stages: 8 h at 50 ºC, 3 h at 80 ºC and 1 h at 100 ºC.

III. Results

The appearance of diatom fouling at the surface of porous UHMWPE has some specific features, which are related, as shown below, to diatom physiology and adaptation mechanisms.

Karayevia amoena

The individuals of this species are known to adopt a sedentary mode of existence, whereby they attach their bottom frustule to the substrate surface using longitudinal raphe and a number of areolae. These diatoms do not undertake significant displacements: as a rule, each alga performs a unique movement per lifetime, namely, following binary (diatomic) division it slides off the surface of the underlying half and moves aside a distance comparable with its own dimension. This type of algae behaviour predetermines the type of colonization pattern – the settlements of these algae are dense and spread in a single layer over a limited area.

It was possible to distinguish three main patterns of colonization:

Pattern 1. Mono-chains of primary colonization: the algae are disposed one-by-one along the axis of movement, with the misorientation angle of 10-45˚, depending on ridges of the underlying UHMWPE micro-relief. Sliding-reflection symmetry is often noticeable. The driving force for structure formation is expansion combined with the detection and adoption of surfaces suitable for planar growth. Finally, there is localization at the ribs and ridges in the UHMWPE relief, as shown in Fig. 1.

Pattern 2. Joined-up chains of secondary (planar) colonization: individual algae cells are disposed in the form of adjacent oblique clusters (Fig. 2), with axes oriented at the angles of 20-50° with respect to the cross axis of the series, with 3-5 cells per series. The neighboring chains may have either co-directional or "herringbone"-like character. The driving force for the formation of such chains is the planar "carpet" colonization of relatively large area over which the cells reside in various spotty scatter formations. Each spot corresponds to a compact group of cells which are approximately uniform in number.

Pattern 3. Annular joined-up chains of extensive colonization with substrate deformation. In this pattern, the cells are disposed at an angle of 10-45˚ to the longitudinal axis of the series, joined tightly along the sides; the driving force the creation of secondary colonization structures with polymer substrate stretching and the attendant increase in the area for colonization. Localization is seen on small planar UHMWPE areas, as shown in Fig. 3.

After reaching the maximum density of in-plane colonization, diatoms begin to transform the underlying surface using their adhesion strength and further increase the number of individual algae in each series, as seen in Fig. 3b.

A spheroidal structure is formed at the final stage of population evolution (Fig. 3b-c) has the appearance of a "pot" with the top opening formed by chains of individual diatoms that line up the outer surface. The diameter of "pots" varies in the range 60-80µm depending on the stage of evolution from planar structure. Accordingly, the "pot' volume amounts to ~300μm^3. The distance between "pots" on the sample surface ranges between 300 µm and 700 µm, with the distribution being relatively uniform.

Halamphora coffeaeformis

Much more mobile than *Karayevia amoena*, diatoms of the species *H. coffeaeformis* are able to move autonomously when searching for a palace of attachment at a distance that may be ten to a hundred times the size of their frustule.

H. coffeaeformis algae arrange themselves along the tops of the "hills" formed by the numerous folds of the scaffold, and along the edges of large caverns. Each algae cell attaches itself to several micro-folds of the substrate (Fig. 4a). The three types of colonies that were observed in the populated samples were: a) individual cells; b) chains of primary colonization, with cells were arranged in diverse clusters with angles of 45-50° made with the axis by individual algae; c) extended compact colonies which arise as a consequence of the planar expansion of the chains. As a rule, these were arranged along the edges of craters present in the substrate relief, as seen in the image in Fig. 4c.

The issue of the diatom-substrate interaction mechanism is of great importance for engineering applications, as understanding this mechanism may serve to guide the process of diatomic colonization on the chosen substrate. The investigation was directed at obtaining insights using FIB-SEM visualization techniques. The attachment of diatom to the substrate may occur via a number of mechanisms:

a) adhesion by means of poly-saccharide 'glue' agents;
b) suction force generated by the diatom cell;
c) mechanical gripping via asperities at frustule surface;
d) a combination of the several of the above mechanisms.

The fracture of the underlying polymer substrate and the formation of a crack at the periphery of frustule attachment (seen on the left in Fig.5) implies significant plastic deformation that is likely to be caused by mechanism (b). Given the yield strength of non-oriented UHMPE of about 20 MPa, the source of high surface tension forces that arise at the diatom-polymer interface must be sought and explained. UHMWPE and bio-silica are chemically inert, so it is difficult to ascribe a significant contribution to the chemical affinity factors. However, one must also take into account the specific electrochemical processes inside the frustule caused by metabolism of algae cell which may be responsible for the observed strong interaction of diatoms with the substrate employed for the purposes of migration and re-attachment by the mobile species *H. coffeaeformis*.

The final aspect of diatom structure investigation addressed in the present study was the use of FIB sectioning and SEM imaging to reveal the internal structure (Fig.5). In the image, the bright white coverage corresponds to the deposited protective Pt coating. The thin (~200nm) bio-silica frustule is seen in section, together with the organism residing inside, occupying ~30% of the inner volume of the exoskeleton. It is interesting to note the appearance of electron reflection in the lower part of Fig.5 that may be associated with the effect of charging of the polymer substrate.

IV. Conclusions

The study presented in this report charts the way to the investigation of colonization of porous polymer scaffolds by different species of diatomic algae. By employing FIB-SEM imaging it has been possible to reveal multi-scale processes that accompany the proliferation of diatomic population, namely, distributed spots of coverage, the structure of clusters and the formation of 'pot'-like arrangements, and some details of the attachment of diatom frustules to the substrate.

Among the whole host of interesting questions that arise in this regard, the one concerning the mechanism of diatom attachment to the polymer is particularly intriguing. In the first instance it appears that the plan of attack should involve quantitative measurement of the attractive forces involved, since this information is likely to be helpful in identifying the physics behind this phenomenon.

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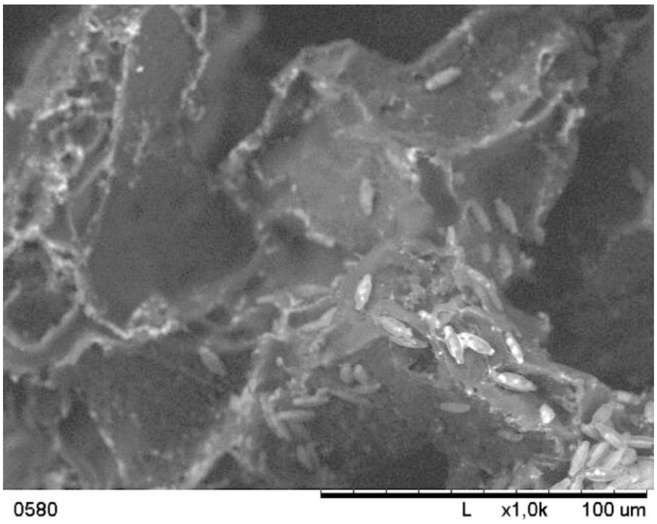

Fig. 1. The pattern of primary colonization (*Karayevia amoena*).

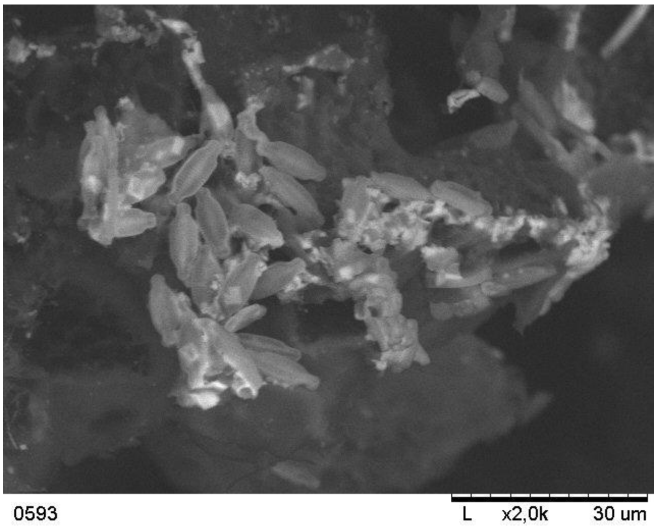

Fig. 2. The pattern of secondary colonization (*Karayevia amoena*).

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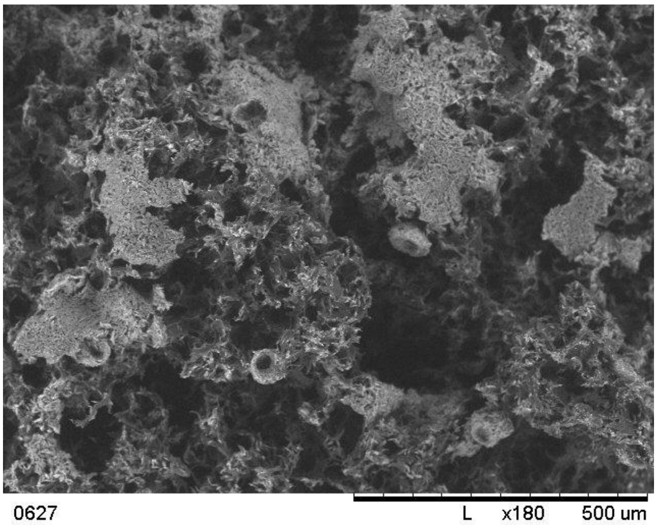

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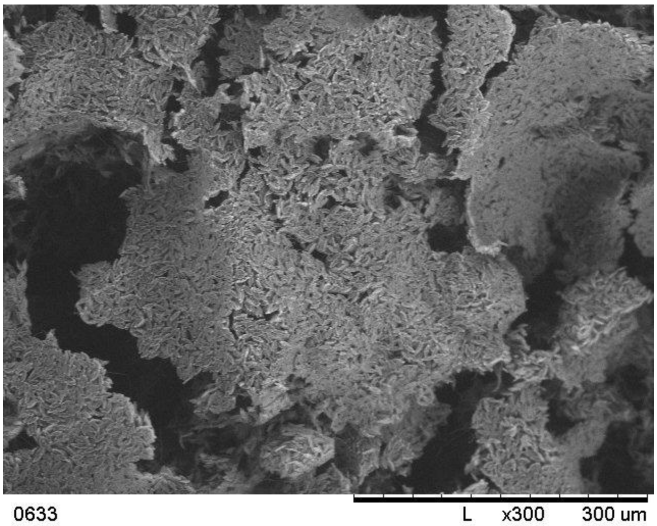

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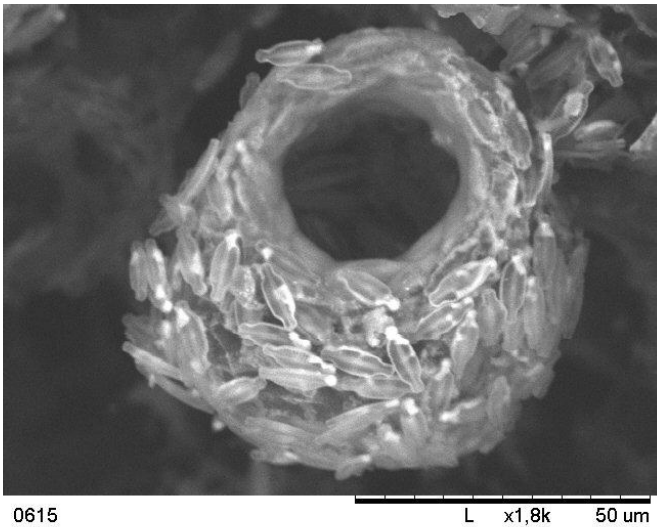
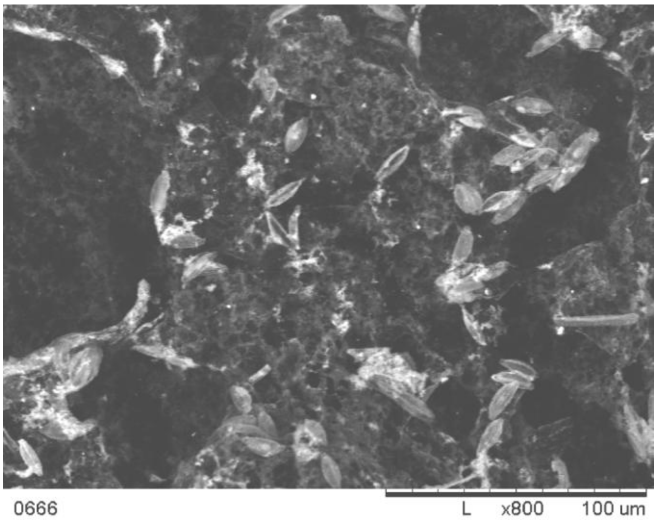


c)

Fig. 3. Ternary colonization of UHMWPE by *Karayevia amoena*. a) general pattern; b) fully colonized plain areas of porous UHMWPE; c) specific ‘pot’-like patterns of ternary colonization.



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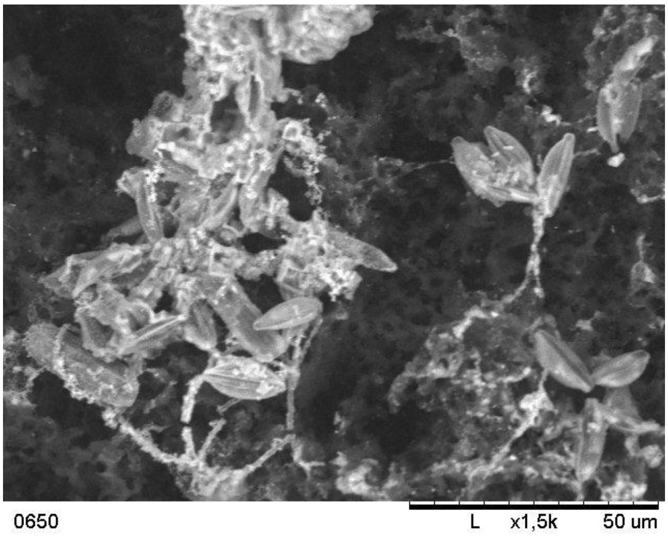

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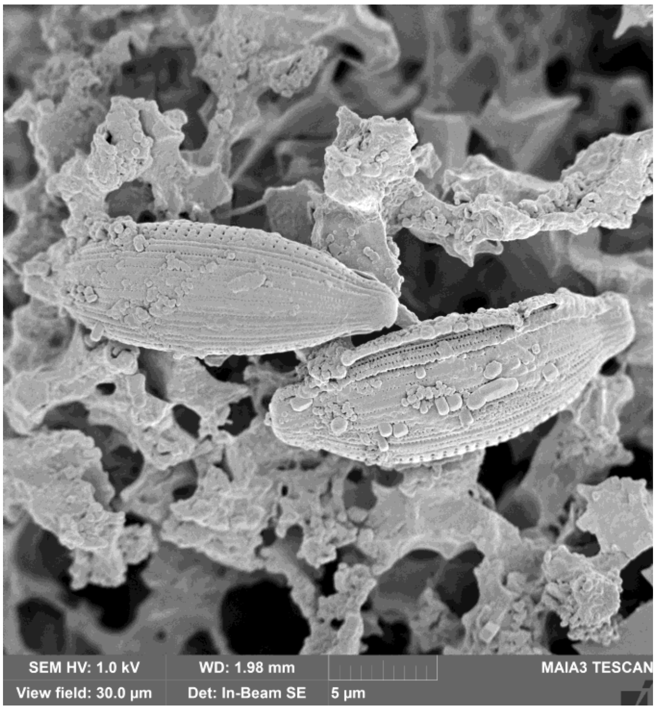

c)

Fig. 4. The patterns of colonization by *H. coffeaeformis*. a) Primary chains, b) compact colonies, c) individual algae cells.

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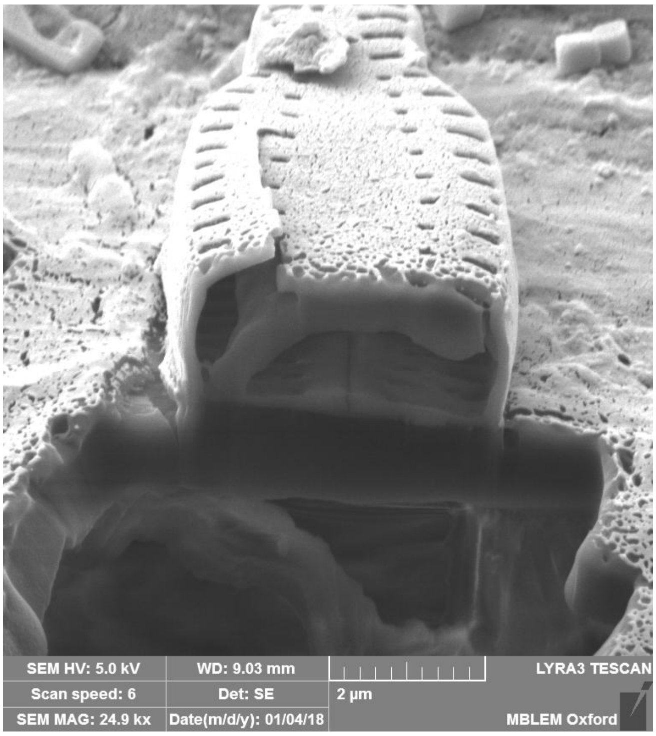

Fig. 5. FIB cross-section of a single *Karayevia amoena* fixed to the UHMWPE substrate.

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