ABSTRACT BOOK OF THE

1ST INTERNATIONAL CONFERENCE ON BIOLOGICAL AND BIOMIMETIC ADHESIVES

9-11 May 2012

School of Dentistry – University of Lisbon

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8h00-9h00: Registration

9h00-9h10: Welcome and short introductory talk of the University of Lisbon and School of Dentistry (Dr. António Vasconcelos Tavares – Vice-Dean University of Lisbon)

9h10-09h20: Welcome talk of the Portuguese Foundation for Science and Technology

09h20-09h30: Presentation of COST Action TD0906 (Dr. Patrick Flammang – President of COST Action TD0906)

SESSION I: CHEMICAL CHARACTERIZATION OF ADHESIVES
(Chaired by Dr. Markus Linder)

09h30-10h10: Keynote talk of Dr. Andrew Smith (USA) – "Multiple metal-based cross-links: protein oxidation and metal coordination in a biological glue"

10h10-10h50: Keynote talk of Dr. Kei Kamino (Japan) – "Biological adhesive, package of wonder"

10h50-11h10: Coffee break

11h10-11h30: Mr. Mathias Grunér (Finland) – "The fungal protein Hydrophobin I as an adjustable glue module for wet bioadhesion"

11h30-11h50: Mr. Meir Haber (Israel) – "Film forming and adhesive properties of algal biopolymers"

11h50-12h10: Dr. Peter Ladurner (Austria) - "The duo-gland adhesive system of the flatworm Macrostomum lignano"

12h10-12h30: Miss Jaimie-Leigh Jonker (Ireland) - "Characterization of the adhesive system of the goose barnacle"
12h30-12h50: Miss Mélanie Demeuldre (Belgium) - "Instantaneous adhesion: the sticky threads of sea cucumbers"

12h50-14h20: Lunch

SESSION II: STRUCTURAL CHARACTERIZATION OF ADHESIVES (Chaired by Dr. Nick Aldred)

14h20-15h00: Keynote talk of Dr. Kathryn Wahl (USA) - "A sticky situation: in situ chemistry and mechanics of barnacle adhesive formation and curing"

15h00-15h40: Keynote talk of Dr. Julius Vancso (Netherlands) - "Bioadhesion and fouling at the nanoscale by Atomic Force Microscopy"

15h40-16h10: Dr. Henrik Birkedal (Denmark) - "Sticking under tension: the mineralized adhesive structure of the mermaid's toenail"

16h10-16h30: Mr. Michael Bennemann (Germany) - "Biomimicry of the adhesive organs of stick insects (Carausius morosus)"

16h30-16h50: Coffee break

16h50-17h10: Mr. Alessio Di Fino (UK) - "Influence of surface charge and surface energy on the adhesive 'footprint' morphology of Balanus amphitrite and B. improvisus cyprids"

17h10-17h30: Miss Francesca Tramacere (Italy) - "What can we learn from Octopus?"

17h30-17h50: Miss Laila Higgins (Ireland) - "Qualitative and quantitative study of spiny starfish footprints using Atomic Force Microscopy"

18h00-20h00: COST Action TD0906 Management Committee meeting

DAY 2 – 10 MAY 2012 (Thursday)

8h30-9h00: Registration
SESSION III: MECHANICAL TESTING OF ADHESIVES AND THEORY
(Chaired by Dr. Stanislav Gorb)

9h00-9h40: Keynote talk of Dr. Manoj Chaudhury (USA) - "Some examples of crack trapping in natural and man-made systems"

9h40-10h20: Keynote talk of Dr. Costantino Creton (France) "Viscoelastic adhesives: stickiness between the fluid and the solid"

10h20-10h40: Dr. Mattias Berglin (Sweden) - "Fimbria mediated adhesion of E. coli to nanopatterned surfaces"

10h40-11h00: Coffee break

11h00-11h20: Ms. Lena Frenzke (Germany) - "Plant glue: Exploring the attachment mechanisms of Peperomia fruits"

11h20-11h40: Mr. David Labonte (UK) - "Insect adhesion: generating high friction with a lubricated pad"

11h40-12h00: Dr. Thomas Endlein (UK) - "Sticking like sticky tape- how tree frogs manage to cling to overhangs"

12h00-12h20: Mr. Thomas Bras (Belgium) – "Adhesion debonding instabilities: transition from elastic interfacial to viscous cohesive instabilities"

12h20-14h00: Lunch

14h00-15h00: Poster session

SESSION IV – FABRICATION OF BIOMIMETIC ADHESIVES
(Chaired by Dr. Aranzazu del Campo)

15h00-15h40: Keynote talk of Dr. Russell Stewart (USA) – "Sandcastle worm-inspired biomaterials"

15h40-16h20: Keynote talk of Dr. Metin Sitti (USA) – "Gecko-Inspired elastomer micro-fibers with spatulated tip endings"
16h20-16h40: Coffee break

16h40-17h00: Mr. Michael Röhrig (Germany) - "How geometry affects dry adhesion - A systematic design study using 3D Direct Laserwriting"

17h00-17h20: Miss Sabine Akerboom (The Netherlands) - "Self-assembled bioinspired dry adhesives"

17h20-17h40: Dr. Zahid Shafiq (Germany) - "Bioinspired underwater bonding and debonding on demand"

17h40-18h00: Ms. Paulina Zietek (Poland) - "Mimicking natural vascular tissue - endothelial cell adhesion on modified polyurethane surface"

18h00-18h20: Dr. Mustafa O. Guler (Turkey) - "Surface-adhesive and bioactive self-assembled peptide nanofibers for bioinspired functionalization of metal surfaces"

20h30: Conference dinner

DAY 3 – 11 MAY 2012 (Friday)

8h30-9h00: Registration

SESSION V – APPLICATIONS OF BIOMIMETIC ADHESIVES
(Chaired by Dr. Willi Schwotzer)

9h00-9h40: Keynote talk of Dr. Alexandre Cavalheiro (Portugal) – "Dental adhesives – clinical problems"

9h40-10h20: Keynote talk of Dr. Lothar Schlösser (Switzerland) - "Transforming scientific results into commercial products"

10h20-10h30: Coffee break

10h30-11h30: Dr. Nuno Silva (UL INOVAR, Technology Transfer Office) - "Patents: practical answers for the practical researcher"

11h40-12h00: Closing remarks
AGENDA OF COST ACTION TD0906 MANAGING COMMITTEE MEETING

1. Adoption of agenda
2. Minutes of last meeting
3. Matters arising
   3.1. New AO
4. Report from the COST Office
   4.1. News from the COST Office
   4.2. Status of Action, including participating countries
   4.3. Budget status, budget planning and allocation process
5. Progress report of working groups (presented by WG leaders)
6. Action planning
   6.1. Annual Progress Conference (preparation and/or feedback from DC)
   6.2. Action Budget Planning
   6.3. Action Planning (including meetings)
7. STSM status, applications (presented by the STSM coordinator)
8. Publications, dissemination and outreach activities
   8.1. Proceedings volume
9. Request for new members
   9.1. New requests
   9.2. Recently approved members
   9.3. Rules for membership approval
10. Promotion of gender balance and of Early Stage Researchers (ESR)
11. Non-COST country participations
12. Web news (presented by the website coordinator)
13. AOB
14. Closing
Dr. Romana Santos (Vice-Chair of COST Action TD0906 and Portuguese delegate in the Management Committee)

Dr. Marise Almeida (Member of COST Action TD0906, co-leader of WG1)

Dr. Maria Manuela Lopes (Member of COST Action TD0906)

Dr. Luis Pires Lopes (School of Dentistry of University of Lisbon)

Cidália Godinho (School of Dentistry of University of Lisbon)

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Dr. Patrick Flammang (Chair of COST Action TD0906 and Belgium delegate in MC)

Dr. Romana Santos (see above)

Dr. Markus Linder (Member of COST Action TD0906, leader of WG1, Finland delegate in MC)

Dr. Marise Almeida (Member of COST Action TD0906, co-leader WG1)

Dr. Nick Aldred (Member of COST Action TD0906, leader of WG, UK delegate in MC)

Dr. Mathias Berglin (Member of COST Action TD0906, co-leader of WG2, Sweden delegate in MC)

Dr. Stanislav Gorb (Member of COST Action TD0906, leader of WG3, Germany delegate in MC)

Dr. Philippe Leclere (Member of COST Action TD0906, co-leader of WG3, Belgium delegate in MC)

Dr. Aranzazu Del Campo (Member of COST Action TD0906, leader of WG4, Germany delegate in MC)

Dr. Willi Schwotzer (Member of COST Action TD0906 co-leader of WG4, Switzerland delegate in MC)
Multiple metal-based cross-links: protein oxidation and metal coordination in a biological glue

Dr. Andrew M. Smith
Ithaca College, Department of Biology, USA

Metal ions provide a powerful mechanism for cross-linking adhesives and other biomaterials, especially in aqueous environments. Metal ions can cross-link polymers directly through coordination, and some metals can catalyze redox reactions that drive the formation of other cross-links. It is likely that many biomaterials depend on multiple metal-based cross-links, involving variations of direct and oxidative mechanisms. The glue of gastropod mollusks demonstrates this complexity well. The terrestrial slug *Arion subfuscus* utilizes both types of interactions to strengthen their defensive glue. This glue contains substantial amounts of calcium, zinc, iron and copper. In addition, there are metal-binding proteins that are unique to the glue that have gel-stiffening activity. These proteins bind to both iron and zinc, and likely other metals. The function of these proteins, and the integrity of the glue overall, depends on metals. One mechanism that appears to play a central role is metal-catalyzed oxidation. Several prominent proteins in the glue are heavily oxidized, and experimental work has provided evidence that the resulting carbonyl groups link with primary amines to form imine bonds. Specific disruption of these bonds decreases glue stiffness significantly. These findings are noteworthy because common amino acids such as lysine can be readily oxidized by metals. While it has been known that oxidation of the rare amino acid 3,4-dihydroxyphenylalanine plays a role in other biomaterials, these results suggest an even broader role for protein oxidation. In addition to oxidative cross-links, metals directly cross-link slug glue, likely through coordinate covalent bonds. Evidence suggests that calcium directly cross-links the gel through interactions with sulfate on polysaccharides. Surprisingly, zinc does not strengthen the gel, though it is present in large quantities in slug glue associated with key proteins, and it is a common cross-linker in other biomaterials. Thus, it may play a different role. Overall, slug glue demonstrates interesting variations on the metal-dependent mechanisms that have been described thus far. Slug glue is also interesting because it is a dilute gel. It typically contains 97% water, and appears to be a modification of the normal
lubricating slime. The ability to convert a dilute, lubricating gel into a strong glue demonstrates the power of metal-based cross-links.

Keywords: Mollusk, metal, oxidation, gel

References:

In nature there exists a variety of diversified adhesives to meet the individual demands of many organisms. An attachment could occur under water or in the air, with fixation or locomotion, with the animal’s body part attached to foreign materials or by joining different foreign materials together. There may be differences in the attachment area’s size and surface cleanliness, different time lengths needed to attain a full strength of attachment and differences in the weight and direction (tensile or shear) to be loaded. These biological adhesives are excellent models from which to learn how to artificially attach materials in water and to obtain information that will be useful to develop general theories in the interface sciences.

The barnacle is a unique sessile crustacean. The animal firmly fixes its calcareous base shell to a foreign material in water via an underwater adhesive called “cement”. When the barnacle grows, its base reaches a few cms in diameter, thus the barnacle cement fixes the calcareous base with an area of several cm² by an adhesive layer with a few microns in thickness. In other words, the barnacle cement could support a much larger area of distinctly different materials with a thinner adhesive layer for more than a year.

The cement is a protein complex whose proteins are very unique among all of the proteins found in public databases. Neither sequence homology to mussel byssal
proteins / tubeworm cement proteins nor modification of an amino acid residue of tyrosine to DOPA, which is a typical and essential one in adhesives of mussel and tubeworm, were found in the barnacle cement proteins. Unique concepts identified in the barnacle cement include contribution of defined 3D-structure of the proteins, involvement of molecular interaction for the curing, and cocktail of different proteins for surface coupling. Although three invertebrates, mussel, tubeworm and barnacle, all encounter the same obstacles in underwater attachment, they have diversified ways to overcome them. They will therefore give different insights on material designs for us.

There have been several motivations in the study on barnacle attachment. Because a barnacle’s attachment causes serious damage to ship services and water uptake in power plant cooling systems, extensive research regarding barnacle adhesive has been motivated by the need to develop anti-fouling technology. In another current, the underwater adhesive has been intrigued because attachment of materials in water is an undeveloped technology, thus research was motivated to learn the material and the principle. Because both concerns have actually opposite directions, researches have been separately carried out. However, two concerns recently are becoming to intersect each other. In this talk, I will summarize what is known in the barnacle cement.

Keywords: Barnacle, cement, protein based materials

References:
Kei Kamino, Chapter 12., In Structural interfaces and attachments in biology, Springer-Verlag, Berlin, Heidelberg, in press

A sticky situation: In situ chemistry and mechanics of barnacle adhesive formation and curing

Dr. Kathryn J. Wahl
Chemistry Division, US Naval Research Laboratory, USA

Proteinaceous secretions are widely recognized to be significant contributors to marine biofouling. The resulting interfacial films can be physisorbed or chemisorbed, and have varying degrees of permanency – they may be highly polymerized and cross-linked, or simply sticky enough to allow surface exploration. Conventional approaches to examining bioadhesive junctions are
forensic in nature – biofouler removal (separating the surfaces) followed by ex situ examination of the adhesive composition and surface morphology. We have tackled a common marine fouler, the barnacle, with in situ approaches and tools that allow us to probe the bioadhesive interface as it develops. From these studies, we have learned that barnacles secrete and cure their adhesive through a multistage process. In this talk, I will present examples of how we have applied a broad suite of in situ microscopy and spectroscopy approaches to reveal how barnacles form and cure their adhesive, as well as its properties and composition. Our in situ approaches include performing temporally- and spatially-resolved microscopy and spectroscopy through adhesive interfaces transparent at UV, visible, IR, and x-ray wavelengths [1]. We have used these and other materials science tools to extend our understanding of the properties and development of the adhesive interface of barnacles. Barnacle adhesion is promoted by both chemistry and structure at multiple scales. Further, the proteinaceous adhesive interface is formed in two steps, with the second process modifying the interfacial chemistry and increasing adhesion twofold. Ultimately, we aim to use this information to develop better antifouling strategies that reduce bioadhesive strength.

Keywords: Barnacle, adhesion, biofouling, FTIR, AFM, tomography

References:

Bioadhesion and fouling at the nanoscale by Atomic Force Microscopy

Dr. G. Julius Vancso
MESA+ Institute for Nanotechnology University of Twente, The Netherlands

Understanding of interfacial phenomena and engineering of surfaces in contact with seawater to control settlement of marine species have benefitted considerably from the use of Atomic Force Microscopy (AFM). AFM can be employed to image substrate surfaces and capture the morphology of substrates at various stages of the fouling process with a resolution from the nanometer to
the micrometer length scales. Simultaneously, at preselected locations, the adhesion of the proteinaceous material deposited by fouling species, such as barnacle cypris, can be assessed by measuring force-extension curves using the AFM based force spectroscopy approach. Finally, the adherence of anti-fouling surfaces can be tested, using chemically modified AFM tip-probes. In this presentation recent progress in the above areas will be highlighted.

Some examples of crack trapping in natural and man-made systems

Dr. Manoj K. Chaudhury
Department of Chemical Engineering, Lehigh University, USA

We will briefly review certain concepts of crack trapping underlying the adhesion of certain natural and man-made systems. After providing examples from our as well as other works, I would discuss the behavior of thin soft films under confinement, in which instability patterns appear at the interface in the form of bubbles or fingers. After discussing the physics behind the morphological pattern formations under different conditions of stress, we will define a critical confinement parameter in terms of the material and geometric properties of the system and discuss how to tune this parameter to control the onset of instability. We will then discuss the roles of these instabilities in the adhesion and friction of thin confined films.

Viscoelastic adhesives: stickiness between the fluid and the solid

Dr. Costantino Creton
Laboratory of Soft Matter Science and Engineering, France

Insects and small animals often use a combination of a complex geometry and a sticky viscous or viscoelastic layers on their feet to be able to adhere to specific surfaces. Some of the situations observed in nature have inspired biomimetic equivalents which have focused more on geometry and mechanics than on material properties.
Yet the field of synthetic adhesives has studied for quite some years the adhesive properties of thin viscoelastic layers. Materials or surfaces which are sticky to the touch must be, not only soft enough to conform easily to rough surfaces, but sufficiently viscoelastic to relax stresses at crack tips and prevent crack propagation at the interface. Such properties are typically obtained in soft viscoelastic solids or in highly viscoelastic fluids. Using a contact mechanics method as a main tool, we will present a review of the differences between viscous liquid and elastic solid adhesion as well as a transition between the two regimes. We will stress recent advances in the characterization of the large strain behavior of the adhesive layer to control the adhesion mechanisms and the final detachment mechanism.

Keywords: Adhesion, rheology, adhesives, fracture, viscoelasticity

References:

Sandcastle worm-inspired biomaterials

Dr. Russell J. Stewart
Department of Bioengineering, University of Utah, USA

Sandcastle worms cobbled together composite tubular dwellings with sand, seashell fragments, and dabs of a multi-part glue. Molecular components of the glue include at least four polybasic proteins (Pc1,2,4,5), a set of polyacidic phosphoproteins (Pc3A,B), polyacidic sulfated polysaccharides, divalent cations (Mg2+/Ca2+), and iron. Sets of oppositely charged components are electrostatically condensed into four distinct types of granules in at least four types of secretory cell types. The most prominent secretory cells, clustered around the cavity of the first three parathoracic segments, contain homogeneous and heterogeneous granules named for their distinct morphologies. The heterogeneous granules contain sub-granules of condensed polyphosphates.
(Pc3A,B) and divalent cations. The heterogeneous granules also contain the polybasic Pc1 and Pc4 proteins as revealed by immunolabeling [1]. The homogeneous granules contain polybasic Pc2 and Pc5 as well as sulfated polysaccharide counter polyanions. The multiple granule types are co-secreted. Shearing during or shortly after secretion ruptures the granules and their contents are partially mixed. Shear forces may be generated by a combination of three mechanisms: squeezing through narrow openings in the surface of the building organ (a strainer), the beating of bundles of paddle-shaped cilia that cover the building organ, or by twisting of the mineral particles as they are set in place. The glue hardens into a foamy load-bearing solid within 30 s after secretion. The sandcastle glue has served as a useful model for the development of synthetic underwater adhesives based on condensed polyelectrolytes (complex coacervates) [2]. The sandcastle worm-inspired adhesives are being developed for repair of soft and hard living tissues [3] General purpose versions of the adhesive designed for the high ionic strength of the ocean maybe useful for undersea applications, including coral seeding for reef restoration.

Keywords: Complex coacervate, sandcastle worm, biomimetic adhesives

References:

Gecko-inspired elastomer micro-fiber adhesives with mushroom shaped tip endings

Dr. Metin Sitti
Carnegie Mellon University, Pittsburgh, USA

Micro- and nanoscale beta-keratin fibrillar structures on the feet of geckos and other climbing insects have been of great interest because they can repeatedly and strongly adhere to wide range of surfaces in various environments [1]. In this presentation, mechanics, fabrication, characterization, and applications of elastomer fibrillar adhesives inspired by these biological foot-hairs will be introduced. Various polymer micro/nano-fiber designs are proposed and fabricated using optical lithography, dip-transfer, and molding based micro/nanofabrication techniques [2-4]. First, adhesion and friction of vertical
polyurethane elastomer micro-fiber arrays with mushroom like tip endings are shown to enhance adhesion and friction as strong as gecko foot-hairs on smooth and rigid surfaces. These elastomer fibers are highly repeatable; their adhesion reduces only around 15% after thousands of attachment-detachment cycles. A water droplet is demonstrated to clean these synthetic fibers fully when they are contaminated by dirt particles. The bare fibers degrade their adhesion under water; therefore, mussel inspired DOPA methacrylate coatings on the fiber tip endings are used to enable improved adhesion strength in such fully submerged conditions. Moreover, it is shown that fibers have reduced adhesion on smooth soft substrates due to reduced equal load sharing. Furthermore, oriented elastomer fibers with angled tips are proposed to enable highly directional/anisotropic friction and controlled adhesion similar to biological foot-hairs. Finally, miniature robotics (climbing robots and medical soft capsule robots) and robotic manipulation applications of these fibrillar adhesives are demonstrated. In addition to these robotic applications, these new gripping materials could have broad applications in sports, space, textile, product design, medical, and packaging industry.

Keywords: Gecko adhesion, polymer fiber mechanics, micro/nano-mechanics, repeatable adhesion

References:

Dental adhesives: Clinical problems

Dr. Alexandre Cavalheiro
School of Dentistry, University of Lisbon, Portugal

This presentation will discuss the latest advances in adhesive dentistry. It will also discuss why it is still difficult to achieve a durable hermetic sealing of the interface tooth-dental restoration, essential to increase the longevity of dental restorations.
New products: 1) that solve an existing problem; 2) that have special properties; 3) that have the potential to create a market; 4) secure the future of a company.

How to get to new products successfully? Starting points can be: 1) Observations: by observation already existing solutions are detected, they must be discovered and exploited; 2) Ideas: on how they are meant here, have no concrete end product in sight; 3) Clear objectives: they already clearly describe the desired properties of the final product.

Most commercial products for daily life run through a development process. Often additionally also a research process. Shall the research and development process be successful? Some important factors are crucial: 1) The correct working mode according to the requirements of the project; 2) a suitable project management.

In the presentation the relationships will be identified how the transforming of scientific results into commercial products can be performed successfully.

Keywords: Research, development, transformation, scientific results, new products, project management
The fungal protein Hydrophobin I as an adjustable glue module for wet bioadhesion

Michel Lienemann, Mathias Grunér & Markus Linder

VTT Technical Research Centre of Finland, Bio- and Chemical Processing, Espoo, Finland

Hydrophobin I (HFBI) is a small (7.5 kDa) and tough amphiphilic protein that is produced by the filamentous fungus *Trichoderma reesei* [1]. HFBI can be readily joined with other polypeptides by genetic engineering and produced in large amounts (upto gram scale) using fungal or plant production systems. These properties as well as its exceptionally high solvent resistance and affinity for apolar surfaces make HFBI an interesting molecule for the modular design of non-toxic bioadhesives. So far, several HFBI fusion proteins have been produced containing catalytic modules (e.g. enzymes) or adhesive protein domains (e.g. biotin-specific avidin and cellulose binding domain). Such HFBI fusion proteins were successfully employed for the manufacturing of biosensors and novel composite materials consisting of hydrophobic graphene and cellulose nanofibers [2]. In these HFBI applications, hydrophobic substrates are covered with protein layers which relies on the ability of HFBI to simultaneously bind other HFBI molecules as well as a hydrophobic substrate. In addition, it has been found that adsorbed HFBI layers can bind other proteins – being the third interaction partner – in a pH-dependent fashion [3]. This observation suggested that charged amino acids could play a significant role in HFBI adhesion and HFBI variants with mutated charged amino acids were produced and characterised in order to test this hypothesis. The presented experiments aimed to clarify the effect of the introduced mutations on the tendency of the HFBI to form oligomers and to associate with hydrophobic substrates. The deduced findings will be useful to extend range of HFBI applications by rational design of its interaction with binding ligands.

Keywords: Hydrophobin, protein adsorption, self assembly, surface modification, biosensor, bioadhesion

Film forming and adhesive properties of algal biopolymers

Meir Haber & Irina Lir

Biota Ltd., Israel

Benthic macro-algae settlement typically involves initial attachment of spores to substratum followed by the secretion of permanent adhesives. It is generally accepted that these adhesives are predominantly polysaccharide-protein complexes, which strengthen over time through a curing process [1]. However, the detailed chemical composition and properties of algal adhesive are generally unknown.

The aim of the present study was to isolate and characterize algal biopolymers, and evaluate their properties, as related to prospective surgical applications. A previously described extraction and fractionation method [2] was used to isolate biopolymers from cultivated red macro-alga Gracilaria Conferta. The chemical composition including protein content, total sugars, hexuronic acid, sulfated sugars, glucoseamines, and total amines of the isolated biopolymers were determined. The main ingredients found were proteins (about 20 %) and polysaccharides (about 70 %). The protein fraction contained a high ratio of glutamic and aspartic acids, RGD (Arg-Gly-Asp), RGD-like (e.g. Arg-Tyr-Asp) adhesion recognition sequences, and neither DOPA (3,4-dihydroxyphenyl alanine) or hydroxyproline were found. The polysaccharide fraction hexoseamines, uronic acid, and sulfate content were 4.5%, 4.6 %, and 0.5 % respectively.

Film forming capacity was studied by using casting method for aqueous solutions. The films possessed tackiness to wet surfaces, high water vapor permeability (2500±103 g•m2/24h at 37 °C), chemotactic (cell attracting) capability (tested by human fibroblast cells and bovine endothelial cells). The film’s mechanical and physical-chemical properties may be modulated within a wide range (tensile strength of 10-70 MPa, elongation at break of 2-40 %, moisture absorption of 25-500 %/24h, and degradation rate of 2-50 %/24h).

Biocompatibility of the formed films was studied. In vitro cytotoxicity test showed no evidence of causing cell lysis or toxicity. In vivo acute intraperitoneum reactivity test in rabbits showed no evidence of significant irritation or toxicity. In vivo acute systemic toxicity test in mice showed no mortality or evidence of systemic toxicity.

Adhesive properties of the isolated biopolymers have been evaluated using lap-shear test according to ASTM 897-95 and 3983-93. Lap-shear adhesion strength
was measured at uniaxially stretching at 80 mm/min, 25±2 °C using Universal Testing Machine Lloyd LRX-Plus equipped with 5 kN load cell and Nexygen 4.0 software package. Three types of prepared algal bioadhesives were tested: untreated algal extract, plasticized, and cross-linked and plasticized. Three types of adherents were used: cellulose acetate, gelatin and mylar. Obtained lap-shear adhesion strengths were in the range of 0.4-0.7 MPa depending on the adhesive tested and the adherent used.

Algal biopolymers and related biomimetic materials may find applications such as surgical adhesives and sealants, enabling non-invasive augmentation of tissues damaged by trauma or surgery [3].

Keywords: Macro-algae, biopolymers, adhesives, films, biocompatibility


The duo-gland adhesive system of the flatworm *Macrostomum lignano*

Peter Ladurner¹, Willi Salvenmoser¹, Roberto Arbore³, Eugene Berezikov², Lukas Schärer³, Paul Kaschutnig¹, Michael Gabl¹ & Birgit Lengerer¹

¹University of Innsbruck, Center of Molecular Bioscience Innsbruck, Institute of Zoology, Innsbruck, Austria; ²Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht, The Netherlands; ³Evolutionary Biology, Zoological Institute, University of Basel, Switzerland

*Macrostomum lignano* (Macrostomida, Platyhelminthes) is an emerging model organism for developmental and evolutionary studies. It is a small (1mm), marine, free-living flatworm that can easily be cultured under laboratory conditions. The natural habitats are sandy beaches where *M. lignano* can be found between the sand grains. *Macrostomum* possesses a duo-gland adhesive system [1] which allows them to adhere and release from the substrate several times within a second. The animals are highly transparent, and exhibit a simple organization of tissues and organs. *M. lignano* is an obligatory cross-fertilizing hermaphrodite that produces eggs throughout the year in laboratory cultures. The *Macrostomum* research community has developed a broad methodological toolbox, including in situ hybridization (ISH), RNA interference (RNAi), transgenics, cell-, tissue-, and organ-specific monoclonal antibodies, energy filtered transmission electron microscopy, and transcriptome and genome sequencing databases. The M.
The goose barnacle *Lepas anatifera* attaches to a wide range of substrates, including organic (wood and skin) and inorganic (glass and plastic) surfaces. Relatively little is known about the permanent adhesive that is produced by barnacles, but the adhesive system appears to be unique compared to other marine animals, such as tubeworm and mussel [1]. The primary aim of the present study was to characterise the adhesive system of *L. anatifera*. The
morphology and glandular chemistry of the adhesive gland were examined using electron microscopy and histochemistry. The adhesive glandular system of *L. anatifera* consisted of a single type of large secretory cell; these ‘unicellular glands’ were isolated from one another and connected to a branched network of drainage canals. Within the gland cells, many secretory granules accumulated around an intracellular canal (ICC), which was the dynamic structure responsible for transporting the adhesive proteins out of the cell. The ICC was able to fuse with secretory granules and envelop cytoplasm containing secretory material. The lumen of the ICC, as well as subsequent larger drainage canals, contained an electron dense, flocculent substance that was not contained within any sort of bound structure. This is quite distinct from the systems used by the mussel and tubeworm, in which distinct adhesive components are physically separated during secretion (from multiple gland types) as well as being separately bound within secretory granules until the adhesive is about to be released from the animal [2, 3]. The barnacle gland cell cytoplasm and the adhesive secretion within the drainage canals were protein-rich, with a pH of around 6. They also contained some carbohydrate.

Once outside the body, the liquid ‘glue’ cures into hard cement on contact with the substrate. The cement is a multi-protein complex. Four of the cement proteins have been characterised [4-7]: they are novel and do not contain the residues DOPA and pSer, which play important adhesive and cohesive roles in other marine adhesives. Two distinct methods are currently being used to examine the cement proteins. The cement was partially solubilised and several protein bands were consistently observed with 1D SDS-PAGE, with molecular weights of approximately 35, 50, 60, 75, 90 and 100 kDa. These bands will be further examined with MS/MS de-novo sequencing. In addition, RNA has been extracted from *L. anatifera* and degenerate primers have been designed, based on the published sequences of acorn barnacle adhesive proteins. Through protein separation, mass spectrometry and PCR methods, we aim to isolate adhesive protein genes and characterise the primary structure of adhesive proteins produced by *L. anatifera*.

Keywords: Adhesive gland; adhesive protein; barnacle; bioadhesion; cement; histochemistry

Instantaneous adhesion: the sticky threads of sea cucumbers

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Some species of sea cucumbers, all belonging to the family Holothuriidae, have peculiar organs playing a role in defence against exterior aggressions, the so-called Cuvierian tubules. When these animals are stressed (e.g. by a predator), they turn their posterior part towards the stimulating source and contract their body, resulting in the discharge of a few tubules. In seawater, these threads lengthen considerably and become immediately sticky upon contact with any object (hence the term “instantaneous” adhesion [1]. The adhesivity of the outer epithelium combined with the low resilience of the collagenous core makes Cuvierian tubules very efficient at entangling and immobilizing potential predators.

In terms of composition, their glue consists of 54% proteins, 36% neutral carbohydrates and 10% of inorganic residues [2]. Of the 3 post-translational modifications usually observed in marine adhesive proteins (hydroxylation, phosphorylation and glycosylation), only the two last ones have been detected on sections of Cuvierian tubules. The use of antibodies revealed the presence of phosphoserine residues in granular cells, one of the two cell types composing the adhesive epithelium. Lectins (proteins that specifically bind to oligosaccharidic structures), on the other hand, never labeled granular cells but galactose- and glucose-binding lectins extensively labeled the mucous vesicles in peritoneocytes (the other cell type constituting the adhesive epithelium) [3].

Two methods were used to characterize further the composition of the adhesive produced by Cuvierian tubules. First, proteins were extracted from glue prints using a protocol developed in our laboratory. Glue prints, which consist of patches of adhesive material left on the substratum after mechanical detachment of the tubule, are indeed extremely enriched in adhesive secretions. After SDS-PAGE separation and in-gel enzymatic digestion, the extracted proteins were analysed by tandem mass spectrometry in order to identify the proteins potentially involved in adhesion. Then, Western blots were performed with anti-
phosphoserine antibodies and lectins to determine which of these proteins are post-translationally modified.

Keywords: sea cucumbers, Cuvierian tubules, proteins, identification, post-translational modifications


Sticking under tension: the mineralized adhesive structure of the mermaid’s toenail

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We investigate the adhesion system (the byssus) of the Anomia simplex also called the jingle shell or the mermaid’s toenail. Contrary to the well-known byssus in the mytilids [1], the Anomia sp. byssus is heavily calcified [2, 3]. In the live animal, the byssus operates in tension and is thus one of the few mineralized tissues dominantly challenged this way.

The hierarchical structure of the byssus is laid bare in detail using a combination of techniques. µ-CT yields an overview of the 3D architecture. Shorter length scales are probed by SEM, EDX, SAXS and WAXS while nanoindentation is used to relate structure to function.

The byssus architecture is found to be highly complex consisting of several inorganic (CaCO3) phases carefully positioned in an organic framework. The structure can be divided into several distinct regions. A thin mainly organic glue region faces the substrate at the very bottom of the structure. Above this a porous region, containing a branched system of tubes extending almost throughout the entire structure. These tubes are lined by an organic sheet in a highly mineralized matrix consisting mainly of Mg-calcite. The central portion and the entire top region towards the musculature consist of lamellae of alternating inorganic and organic layers. The organic lamellae are continuous throughout the byssus and continue into the musculature thus providing a mechanism for interfacing muscle and substrate.
We discuss the byssus architecture in relation to its functional use in the animal.

Keywords: byssus, bivalves, *Anomia simplex*, structure, mechanical properties


**Biomimicry of the adhesive organs of stick insects (Carausius morosus)**

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Adhesive organs enable insects to cling to various substrates even during rapid locomotion. In this process a very fast but reliable change of adhesion and detachment is realised. Some insects are actually able to resist extreme pull off forces equivalent to more than 100 times of their own body weight [1,2]. To reveal the detailed underlying mechanisms of this impressive performance we analysed the ultrastructure of the smooth adhesive organs of the stick insect *Carausius morosus*. Fundamental for a good adhesion seems to be a very thin and elastic terminating membrane, which can adapt to lowest unevenness of substrates. In *Carausius morosus* this membrane is composed of the about 225 nm thick epicuticula and the superimposed about 24 nm thick cuticulin layer [3]. Underneath these membranes the adhesive organs of stick insects show a fibrous structure forming the procuticula [4]. In this layer about one µm thick fibres originate at the subjacent endocuticula and branch several times treelike towards the surface [3]. The finest branches have a diameter of about 105 nm and are connected very closely packed to the epicuticula. This fibrous construction supports the very thin epicuticula without preventing its flexibility necessary for a good adaption to the substrate. Furthermore the treelike branching construction enables the adhesive organs to conform and adhere to substrates with roughness at different length scales. The fibres located in the procuticula are orientated in an angle of about 15° at their origin to about 57° at their terminal end. This construction let react them more elastically than orientated perpendicular to the surface and perhaps permits possibilities for an easy detachment. During the detachment of the adhesion organ the fibres might be used as a lever arm to lose contact. High speed videos of the detachment process suggest this hypothesis. The fibrous construction of the adhesive organs has been transferred on a Finite
Element model to analyse further functions of the fibres. For example, the fibres could improve the moulding of the substrate by pushing the ends of the fibres into surface irregularities. Based on the found mechanisms for the adhesion of stick insects we are developing a technique to fabricate an artificial adhesive material, which shall show high adhesion forces on substrates of different surfaces roughness and which can easily be detached if desired.

Keywords: bionics, insect cuticula, functional morphology, finite element simulation


Influence of surface charge and surface energy on the adhesive ‘footprint’ morphology of Balanus amphitrite and B. improvisus cyprids

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Macrofouling of anthropogenic structures is considered to be one of the biggest challenges currently facing the maritime industry, due to the significant economic and environmental impacts associated with the process. Studies of biological adhesives of marine origin, and particularly those of barnacles, is therefore an important area for research. The deposition of the temporary adhesive (footprint) material, used by barnacle cypris larvae during surface exploration, has been investigated using imaging ellipsometry to better understand the interaction of the adhesive proteins with different, well-defined surfaces. In this study we selected two species of barnacle, Balanus amphitrite and Balanus improvisus, which showed different preferences relating to surface chemistry in previous settlement tests. Self-assembled monolayers (SAMs) with CH₃-, OH-, COOH- and N(CH₃)₃+- terminations were used as settlement substrata to measure the diameter, thickness, area and volume of the proteinaceous material secreted by
exploring cyprids – thus inferring the bulk physicochemical characteristics of the material. Briefly, high thickness and low spreading values were found on positively charged surfaces, on which the lowest percentage of settlement was also observed for both species. This may suggest that the positive surfaces have low affinity for the cyprid adhesive thus repelling settlement. In addition, thickness values were low on the CH3 SAMs and spreading was significantly greater, indicating high affinity and perhaps alluding to an adhesive hydrophobic in nature.

Keywords: *Balanus amphitrite*, *Balanus improvisus*, footprint, imaging ellipsometry, SAMs, adhesion.

What can we learn from Octopus?

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In robotics reliable reversible attachment mechanisms can be used both for robot locomotion in unstructured environments and for objects manipulation. One of the most common solutions used in these fields is the suction cup. This device is used for a wide range of purposes for its industrial-strength reliability, its excellent grip (up to 1 atm) [1], for being easy to use, and eventually for its use in delicate applications (applications that needed to involve soft parts instead of hard ones, e.g. glass handling). However, suction cups show three main drawbacks that limit their versatility as an adhesion device, specifically: low efficiency, necessity of a vacuum pump (periodic maintenance), and need for a smooth surface [2]. These drawbacks may be overcome or, better, may be completely absent in natural suction cups (like, for instance, octopus suckers), which also show high efficiency to perform suction by means of strategic muscular arrangement and can work on almost any non-porous surfaces, in addition to the properties identified for the corresponding artificial ones. In particular, we identified octopus suckers as a key biological model, because of their flexibility, dexterity, and capability to generate large forces on different substrates. Octopus arms, in fact, can perform a number of complex movements, and their capabilities are increased by suckers, which work in distinct ways, and
perform a remarkable variety of functions, such as in locomotion, in anchoring the octopus body to the substratum while holding on to prey, in manipulating small objects, etc. [3]. For this reason, we investigated the morphological and physiological features of this natural adhesion solution, in order to collect new design criteria for innovative bio-inspired adhesion devices. In detail, we carried out four different analyses (CryoSem, Histology, Ultrasonography, and Magnetic Resonance Imaging); then we merged and compared the data in order to have a complete view of each aspect of this amazing natural solution. In accordance with the information gathered, we designed a CAD model of the octopus sucker, and we built the first soft passive prototypes. These prototypes were made with silicone (Dragon-Skin and Ecoflex 00-30 from Smooth-On, USA), and they were tested by means of an ad hoc adhesion setup with an external actuation. We demonstrated the capability of these prototypes to attach in wet conditions to different kinds of substrates, in terms of materials and roughness. Our results showed that, for instance, a suction cup prototype with an outer diameter of 2 cm and a weight of 2 g, has a load capacity of almost 8 N [4], by applying a pressure difference of 10^5 Pa. The experimental results achieved provided us with reference values for the suction strength of the artificial device. The obtained data will be taken into consideration for the design of the actuation mechanisms to be embedded into the octopus sucker-like suction cups. Future work will be focused on developing an actuated artificial device, in order to mimic the full capability of the biological model.

Keywords: Adhesion, suction cup, sucker, octopus, wet

Qualitative and quantitative study of spiny starfish footprints using Atomic Force Microscopy

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Due to their inherent ‘sticky’ nature, and complex, heterogeneous composition, biological adhesives are often challenging samples for investigation with Atomic Force Microscopy (AFM). When working with starfish tube foot adhesives, the tiny quantities secreted by individual tube feet furthers the difficulty for AFM imaging and force measurements. Despite these challenges, it is possible to achieve desirable, nanoscale images of starfish adhesive under physiologically-relevant conditions with little to no sample preparation. Under the same conditions, quantitative nanomechanical measurements are also possible.

Starfish are marine invertebrates belonging to the Echinodermata. The starfish is a highly mobile, benthic animal, which attaches to the seafloor using temporary adhesives secreted from their locomotory organs, the tube feet [1]. To detach, they secrete a deadhesive, leaving the adhesive secretion behind on the surface as a footprint [2].

In previous AFM studies, the starfish footprint has been found to be secreted as a homogeneous film with an overlaid meshwork characterised by a hexagonal pattern [2]. Quantitative force measurements have not yet been reported for starfish footprints.

Here we present AFM data for the spiny starfish, *Marthasterias glacialis*. High-resolution images of freshly secreted *M. glacialis* adhesive, will be presented together with quantitative data for the nanomechanical properties of the adhesive. The goal of our analysis is to elucidate the underlying mechanical mechanism for adhesive strength of this underwater adhesive, particularly in relation to locomotive behaviour.

Keywords: Starfish; footprints; adhesives; AFM; nanoscale imaging; nanomechanics.

**Fimbria mediated adhesion of E. coli to nanopatterned surfaces**

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It is generally believed that type 1 fimbria of E. coli is an important virulence factor. It seems that E. coli uses the Type 1 fimbria to attach to host cells, e.g. epithelial cells in the urinary tract. However, a topic of discussion has also been the involvement of fimbriae for surface adhesion in general. In order to investigate the effect on the surface nanoarrangement on the E. coli attachment we prepared two different surfaces (a) one with 10 nm hydrophobic patches surrounded by non-fouling PEG. The separation between the patches increased linearly from 5 to 50 nm from each other on a 1 cm long test surface. (b) The second surface was a negative of the first, thus having 10 nm non-adhesive PEG patches surrounded by hydrophobically modified surface. Again the separation of the patches increased linearly along the test surface. These two configurations can be described in terms of (a) gradient in number of hydrophobic patches and (b) gradient in size of hydrophobic patches. The general methodology for preparing chemical patterns in the sub 100 nm regime will be discussed. The adhesion/desorption of E. coli as measured in a laminar flow chamber on these two surface configurations was utterly different. These results will be discussed in terms of adhesive catch-bond mechanisms that can take place in specific adhesive structures such as Fimbria in relation to bioadhesives in general.

Keywords: Nanopatterning, bacterial adhesion, fimbria


**Plant glue: Exploring the attachment mechanisms of Peperomia fruits**

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Fruits of epiphytic Peperomia Ruiz & Pav. (Piperaceae) plants release a sticky secretion on their surface which is most likely an adaptation to zoochory, addressing animals as dispersal vectors. Aim of the current PhD project is to examine the functional and evolutionary importance of fruit attachment in the
plant genus *Peperomia*. We are exploring the adhesive and chemical properties of the sticky secretion with regard to seed dispersal ecology and potential biomimetic approaches. Therefore, pull-off force measurements according to Voigt and Gorb [1,2] and qualitative cryo-scanning electron microscopic analyses have been performed on the *Peperomia* fruits. According to our observations, the adhesive shows viscoelastic properties resembling those of pressure sensitive adhesives (PSA). The fruits remain sticky after washing them with water, ethanol, and even acetone. Although ethanol and acetone treatment cause dehydration symptoms at the fruit surface, these solvents do not affect the stickiness of the adhesive secretion, but interestingly increase its capability of rehydration. The *Peperomia* adhesive seems to be highly resistant to dry environment, and may be subsequently rehydrated, resulting in pull-off forces similar to those measured of freshly collected fruits. We will carry out further biomechanical and biochemical investigations in order to gain a comprehensive understanding of the *Peperomia* adhesive’s nature and its bio-inspirational value towards the development of innovative, universal adhesives for dry and moist environments.

Keywords: fruit attachment, plant adhesive, pull-off force, epiphyte


**Insect adhesion: generating high friction with a lubricated pad**

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Many insects are astonishingly agile animals that can climb rapidly on vertical and inverted substrates. On smooth surfaces, they employ adhesive footpads to support their body weight. These structures are either smooth, soft cuticular pads or dense arrays of adhesive hairs [1]. Despite this difference in morphology, the surface contact for both types of pads is mediated by a thin film of adhesive secretion [2-5]. Although such a secretion might be expected to act as a lubricant, measured friction forces are generally larger than adhesive forces, and even a considerable static friction is present [6,7]. How do insects produce static friction with a “wet” contact? In insects with smooth pads, the adhesive secretion has been reported to be a water-in-oil type emulsion [8,9]. To clarify the function of
this secretion, we investigated the influence of water absorbency on friction forces generated by insects with smooth and hairy adhesive pads. Attachment forces for stick insects and cockroaches (smooth pads) were significantly reduced when measured on water-absorbing surfaces, but we found no such influence for flies and beetles (hairy pads). This result suggests that friction forces result from different structural properties for the two pad designs: Insects with smooth pads appear to increase friction forces via the two-phasic composition of their adhesive secretion. By contrast, hairy pads may have an inherently larger shear resistance based on the increased overall length of the fluid-surface contact line, arising from contact splitting. While a surface tension-based model predicts the forces of the two hairy-pad representatives reasonably well, quantitative predictions are difficult for insects with smooth pads, because the detailed physical mechanisms underlying friction forces are still unclear. Our results suggest that the two-phasic secretion provides a mechanism to compensate for the shorter fluid-surface contact line in smooth adhesive pads owing to the absence of contact splitting.

The results of our study indicate that insects have evolved two different ways to enjoy the benefits of a “wet” adhesive system (such as increased contact area on rough surfaces and protection against wear), while maintaining the ability to generate static attachment forces.

Keywords: wet adhesion; tribology; biomechanics; emulsion; attachment


Sticking like sticky tape- how tree frogs manage to cling to overhangs

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Tree frogs use adhesive pads on their fingers and toes to clamber around in the canopy. Additionally, their long limbs help them to bridge larger gaps between
branches and leaves. Previous research has shown that many tree frog species change from a crouched resting posture, where the limbs are closely tucked to the body, to a sprawled posture with extended limbs when clinging on to steeper inclines, e.g. vertical or overhanging slopes.

We investigated this change in posture in White’s tree frog (*Litoria caerulea*) by measuring the ground reaction forces of their limbs on a rotatable platform. This consisted of 24 3D-force transducers that completely covered the surface of the platform and allowed force measurements from all four limbs simultaneously. Hand and foot positions were digitized from a synchronized video recording for various inclines.

The change in posture is triggered by the increasing detachment forces on their adhesive pads caused by a steadily increasing slope of the platform. By spreading out their limbs sideways, the frogs were able to use opposing feet to generate large lateral forces directed towards the body of the frog. Reconstructing the angle between the resultant force vector (the vectorial sum of all forces acting on a limb) and the platform showed that this posture led to smaller limb/platform angles than those generated by a frog in a crouched posture. This is clearly beneficial as peeling theory (as applied to a sticky tape) predicts that the shallower the angle of the resultant force vector, the greater the resistance to peeling. Leg spreading is thus a strategy for maintaining a grip on overhangs where peeling of the pads would result in a fall.

**Keywords:** tree frog, ground reaction forces, adhesion, friction, peeling model

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**Adhesion debonding instabilities: transition from elastic interfacial to viscous cohesive instabilities**

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We present here a study of debonding instabilities in elastomers in response to the variation their viscoelastic properties. We use of a model elastomer, polydimethylsiloxane (PDMS), that is peeled off a solid substrate to observe the evolution of debonding instabilities in confined systems. The viscoelastic PDMS properties were modified by changing the amount of cross-linkers leading to the transition from a pure elastomer until the limit of a viscous fluid. By measuring amplitude, wavelength and critical confined thickness, our results show a
debonding mechanism in elastic regime which starts with bulk fingering instabilities and then evolve to interfacial fingers. This elastic mechanism remains a classical interfacial debonding in three successive steps: (i) a bubble cavity appears in the bulk of the film, (ii) a crack initiates from the cavity and propagates within the film and (iii) the crack propagates to the surface where it exhibits a reproducible morphology of measurable magnitude. In contrary, the viscous regime is characterized by a cohesive fingering instability with a periodic appearance and disappearance, in relation with morphologies observed in Saffman-Taylor instabilities.

Raphaël and coworkers have suggested recently a linear relation between fingering wavelength, Lambda, and film thickness, h, in elastic regime, such as Lambda≈ 4h [1]. In this communication, we will show a large variation of the numerical prefactor as a function of the viscoelastic properties of the debonding material (from pure elastic to highly viscous), which demonstrate that this scaling law has its origin in the material rheological properties. Interestingly, our results obtained in viscous regime indicate also that the speed dependence, which is a key parameter of the Saffman-Taylor instability, do not influence the fingering wavelength, suggesting another mechanism.

Keywords: Adhesive film; Fingering instabilities; Solid substrates


How geometry affects dry adhesion – A systematic design study using 3D direct laserwriting

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We present a design study of gecko-inspired hierarchical structures with dimensions down to the nanometer range. By using 3D direct laserwriting [1] as fabrication technology, we were able to match the elastic modulus and the length of the gecko’s setae very closely. Since 3D direct laserwriting allows for the fabrication of arbitrary micro- and nanostructures, this technique is perfect for design studies and the variation of particular design parameters. Measuring the adhesion by atomic force microscopy with colloidal probes, we systematically
studied the effect of several design parameters like density, aspect ratio or amount of hierarchy levels. Additionally, due to the enormous flexibility of 3D direct laserwriting we were able to examine the influence of tip shape and tilting of the gecko-inspired structures with respect to their dry adhesion performance. In this way, we show that hierarchy is indeed favorable for artificial gecko-inspired nanoscale dry adhesives fabricated with stiff materials.


Self-assembled bioinspired dry adhesives

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The key strategy in many natural attachment systems is the incorporation of patterns, i.e. fibrillar surfaces or subsurface structures. Recently, surfaces patterned with pillars have been developed for reusable adhesion. These structures mimic the fibrillar structures found on gecko feet, which give the lizard the ability to run up and down any kind of surface. However, arrays of simple vertical micropillars do not replicate enough features of the biological systems to give the desired adhesion and the importance of the fibril shape was pointed out (e.g. mushroom or spatula shapes). Furthermore, patterns with small dimensions are often observed to be superior to those with a coarser scale, stimulating research towards complex and finer structures, closer in design and performance to biological systems.

In this work we developed nanofabrication processes based on self-assembly, which allows the fabrication of polymeric nanosized fibrillar arrays with complex 3D structures, hard to achieve with top-down methods. The fabrication of inverse colloidal crystals resulted in hour-glass shaped pillars. Theoretical and experimental data show that this particular fibril size and shape is interesting for reversible adhesion.

Keywords: dry adhesion, bioinspired, self-assembly, nanopatterning

Bioinspired underwater bonding and debonding on demand

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Adhesion underwater, self-healing adhesives that stop catastrophic failure during use, and bonding/debonding on demand are three major questions in adhesion science and technology that are only partially resolved with current gluing systems. Mussel Adhesive Proteins, which constitute high contents of 3,4-Dihydroxyphenylalanine (DOPA) residues represents fascinating example of underwater adhesion with self-healing properties. DOPA-inspired polymers containing catechol side groups with improved adhesion in wet environments have been reported. The self-polymerisation and metal chelation capability of catechol rests allow crosslinking of the polymer chains and reaction with inorganic materials. Here we report on the use of Nitrocatechol-derivatives which allow light-triggered debonding. Nitrodopamin (ND) was incorporated to polymer chains. The presence of o-nitrophenyl ethyl moiety allows photocleavage of ND-crosslinked polymer matrix and, thus debonding of the system. Our results demonstrate that ND can be used as biocompatible crosslinkers to form underwater self-curing, self-healing, surface-reactive and photodegradable materials.

Keywords: Nitrodopamine, underwater adhesion, self-healing hydrogel, light triggered debonding, photo-cleavage


Mimicking natural vascular tissue - endothelial cell adhesion on modified polyurethane surface

Paulina Zietek, Beata Butruk & Tomasz Ciach
Presented work was undertaken to elaborate a repeatable method of fabrication of a material that could be used in various blood circulatory support devices, e.g. artificial heart and its parts or vessels implants. The work involves chemical modification of already existing synthetic polymer - polyurethane - with evaluation after each step and cultivation of human endothelial cells on final material surface in order to indicate cells adhesion, growth and proliferation.

Among other polymers used in cardiosurgery polyurethane is considered to have the best mechanical properties and acceptable biocompatibility [1,2]. However, it has been noticed that during long-time implantation vascular implants made from polyurethane undergo surface corrosion caused by moiety and blood cells activity. As a result, an implant is likely to lose its mechanical properties and can become leaky [2,3]. Moreover, the presence of artificial material that contacts blood can trigger blood clots formation [4-7]. Hence, polyurethane surface has to be modified so the final product would mimic a natural vascular inner tissue, which is resistant to these unwanted phenomena. The most advanced method of such modification is to cultivate endothelial cells on the polymer surface. Endothelial cells exist in blood vessels and provide essential features, such as protection from formation of blood clots (thrombosis) [8,9].

The aim of presented work is to enhance endothelium growth by immobilization of protein - collagen - onto polyurethane surface. Collagen can be bounded by cell receptors - integrins [10] what causes a cascade of internal signals that lead to cell adhesion [11,12]. A three-step chemical method of immobilizing collagen onto polyurethane surface was proposed. In the first step chemically inert polyurethane surface was activated with the use of photooxidation. Then the surface was enriched with carboxyl groups using acrylic acid grafting. In the last step obtained carboxyl groups reacted with amino groups from collagen. A set of parameters such as reaction time, temperature and substrata concentration were tested at each step. Obtained materials were analyzed and used as a scaffold in endothelial cell culture. Studies revealed most suitable parameters of chemical modification of polyurethane. Final materials were non-toxic to endothelial cells and promoted cells proliferation and growth. Also, there was an expected change in cells morphology due to interaction with collagen.

To summarize, polyurethane modified with collagen improves endothelial cells adhesion. As collagen was successfully introduced onto a polyurethane surface, the procedure of three-step chemical modification could be applied to different peptides or proteins. The whole process does not involve harsh conditions or complex equipment, therefore it is suitable to be utilized on a large scale.
Keywords: endothelium, polyurethane, bioactive surfaces, proteins immobilization


Surface-adhesive and bioactive self-assembled peptide nanofibers for bioinspired functionalization of metal surfaces

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Integration of metal-based implants into the existing tissue is a major problem. Herein, we demonstrate biofunctionalization of metal surfaces through mussel-inspired adhesion mechanism conjugated to self-assembled peptide nanofibers in order to overcome biocompatibility issues. Dopa conjugated peptide nanofiber coating was used along with bioactive peptide sequences for specific cell-materials interactions on metal surfaces. Dopa-mediated immobilization of bioactive peptide nanofibers on metal surfaces created a bio-specific interface between cells and the metal substrate. This biofunctionalization strategy can be extended into various surface immobilization systems owing to the versatile adhesive properties of Dopa and the ease of ligand conjugation to peptide amphiphile molecules.

Keywords: Dopa, peptide, nanofiber, bioactivity

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Patents: practical answers for the practical researcher

Nuno Silva

UL Inovar - Knowledge Transfer Unit of the University of Lisbon, Portugal

Patents are sources of technical information, a supplemental form of publication, a networking tool to get connected to other researchers at universities and companies, a way to translate ideas into beneficial products and processes, and a source of funding. They are very important in the fields of chemistry, life-sciences, and, of course, Biological and Biomimetic Adhesives. This workshop will deal with practical aspects of getting patent protection, licensing patents and other related topics.
1. Bacterial adhesives

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Currently, there is an increasing interest on bio-based and bioinspired materials from both the institutional and industrial points of view, which is reflected by the increase of bioplastics production [1]. However, the use of these biomaterials seems to be mainly focused on packaging and food and agriculture sectors.

There are in nature several situations in which organisms make use of adhesion mechanisms, even in adverse conditions where conventional synthetic adhesives would fail. This is the case of gecko [2], flies, beetles and spiders [3], where adhesion occurs due to the complex hierarchical structure of their feet, as well as molluscs and crustaceans [4], web spiders [5] and microorganisms [6], where adhesion mechanisms are mediated by extracellular adhesive proteins.

In the case of microorganisms, cells adhere to each other and/or to a surface by means of the formation of biofilms, where they are frequently embedded within a self-produced matrix of extracellular polymeric substance [7]. This extracellular substance has been considered by the authors of interest in the search of new biobased adhesives.

This research focuses on a protein from Bacillus subtilis, a Gram-positive, non-pathogenic and biofilm-forming bacteria. According to a sequence alignment analysis (BLAST), this protein shows high percentage of homology with sequences of proteins with known adhesive function. Furthermore, in a previous work, the authors have confirmed the involvement of the selected protein in biofilm formation mechanisms of Bacillus subtilis by means of a bioadhesion test, as well as by SEM and CLSM [8].

In this work, M15 Escherichia coli strain has been used as heterologous expression system to express the protein of interest. The adhesive protein under study has been subcloned into a commercial expression vector (pQE60) inducible by IPTG that incorporates a six-histidine tag to the protein at its carboxyl terminal. This tag favours its subsequent purification by affinity chromatography by using a matrix system of nitrilotriacetic acid nickel resin (Ni - NTA).

Once purified the protein, its suitability for its use as polymeric base in adhesive formulations has been assessed. Properties studied include average particle size,
wettability, surface energy, thermal properties and thermal stability, among others.
As a result of this study, it has been concluded that the protein could be explored as a temperature-reactivable adhesive. Furthermore, it exhibits good wettability and certain adhesive capability that can be improved through formulation.

Keywords: biofilm, adhesive, protein, assessment, Bacillus subtilis


2. ATR-FTIR study of the bio-adhesive produced by the seaweed Hormosira banksii

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Classical adhesives are limited by a number of drawbacks such as poor biodegradability, inadequate performance under wet environments and low applicability to biological systems. Adhesives inspired by marine organisms such as seaweed can overcome these limitations and address new perspectives in biomedical and biosensor applications. However, adhesives produced by seaweed are not well characterized and contradicting results are reported in the literature [1,2].

Hormosira banksii has been chosen as an ideal candidate to shed light on the functionalities responsible for surface attachment of seaweed. In fact, H. banksii is distinguished from other less developed seaweed because of the presence of male and female plants which, during their reproductive season, release aploid gametes. When the egg is fertilized by a sperm a diploid zygote containing all genetic information is formed, i.e. the first cell of a new developing plant. Immediate surface attachment is of paramount importance for the newly formed
zygote to ensure its survival in the marine environment, therefore secretion of the glue is one of the first processes triggered after fecundation.

Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectroscopy is a non-invasive and in situ technique that allows the detection of chemical functional groups, conformational changes and adsorption reactions in the proximity of a surface. The ability to run experiments in aqueous environments constitutes a major advantage of this method as physiological conditions similar to that of the natural environment are easily attainable.

In order to investigate the components of the bio-adhesive produced by *H. banksii* plants, ATR-FTIR adhesion experiments have been performed with fresh eggs and sperm as well as with fertilized zygotes using filtered seawater as the aqueous medium. In addition, experiments in dry conditions have been carried out to understand the overall composition of the biological material investigated. Thanks to the comparative analysis of the spectra obtained from the sexual gametes and the fertilized zygotes, new information on the functional groups involved in zygote attachment has been extracted.

Also, SEM and zeta potential measurements have been carried out to determine the morphology and external electric charge of the living samples, providing additional information on the changes occurring after fertilization.

The various results collected will be discussed and new conclusions on the formulation of the glue produced by seaweed will be put forth.

Keywords: Seaweed, ATR-FTIR, bio-adhesive, *Hormosira banksii*, spectroscopy, gametes and zygotes


3. Unique adhesive system in barnacles

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The goose barnacle Lepas anatifera attaches to a wide range of substrates, and is one of the most notorious biofoulers. Wet adhesion in mussels and tubeworms relies on the post-translational modifications dihydroxyphenylalanine (L-Dopa) and phosphorylated serine (pSer) for adhesive and cohesive function[1, 2]. The
The adhesive system of barnacles appears to be unique compared to the wet adhesion of mussels and tubeworms [3]. The objective of this study was to describe the adhesive glands in the goose barnacle Lepas anatifera, with emphasis on the structure and chemistry of tissues involved in adhesive production. The morphology and glandular chemistry of the adhesive gland were examined using electron microscopy, histochemistry and immunohistochemistry. The adhesive of L. anatifera is produced by single type of large secretory cell; these ‘unicellular glands’ were isolated within the stalk; each secreted the adhesive product into an intracellular canal, which then led into a series of drainage canals. Within the gland cells the components that form the adhesive were packaged into secretory granules, however the adhesive components were no longer separated after they moved into the intracellular canal. The lumen of the ICC, as well as the larger drainage canals, contained an electron dense, flocculent substance that was not contained within any sort of bound structure. Such a phenomenon is incredibly distinct from the secretion process in the mussel and tubeworm adhesive systems, in which adhesive components are separated, being produced in different glands and bound within secretory granules until the adhesive is about to be released from the animal [4, 5]. There was no evidence of L-Dopa or pSer in the glue gland of the barnacle, the adhesive that was observed within the drainage canals or the hardened adhesive at the tip of the stalk.

The differences that exist between the barnacle adhesive system and that of other underwater gluing organisms, such as the mussel and tubeworm, indicate that there is a unique molecular bonding system at work in barnacle adhesive.

Keywords: Adhesive gland; adhesive protein; barnacle; bioadhesion; cement; histochemistry

4. The organic matrix in the calcified byssus of Anomia simplex

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Bivalve adhesion involving byssi is best known from the mytilids that employ protenaceous threads. In the anomiids on the contrary, the byssus is highly mineralized by CaCO3 (over 90 wt%)1. Not much is known about the organic part of the byssus that functions as the actual adhesive interface and as the link between mineralized byssus and musculature. The byssus consists of a lamellar
part, which forms the interface to the musculature, a macroporous part, and the actual adhesive interface towards the substrate termed the glue layer. In this study, the organic matrix of the calcified byssus was investigated. The byssus was demineralized by either EDTA or acetic acid. After removing calcium carbonate, a sophisticated framework made of β-chitin and proteins was revealed containing transparent lamellae and a brown basal plaque. To understand how the byssus adheres to a substratum, several characterizations of the organic materials were carried out. The organic matrix rich regions of the byssus are enriched in sulfur. To help identifying which state of sulfur is present, groups that are anionic at pH 2.4 were mapped by staining with the cationic dye alcian blue. The lamellae are strongly stained whereas the region between lamellae and glue layers is less stained. Together with SEM-EDX data, it is suggested that the lamellae contain sulfate groups while sulfur species other than sulfates are in the macroporous region. Interestingly, the substratum (shell) to which the byssus is attached is also stained to a staining depth of 100 μm – 200 μm. This leads us to suggest that the glue penetrates into pore space in the substratum for adhesion. Protein extractions and analyses of the calcified byssi were also carried out. The detection of proteins with aromatic amino acids through UV-Vis spectra implies the existence of adhesive materials (e.g. DOPA) in the extract. By SDS-PAGE, proteins with molecular weights of approximately 50 kDa and 250 kDa were observed. Further characterization of extracted proteins and XPS study of the oxidation states of sulfur in the byssus is ongoing in our lab.

Keywords: byssus, chitin, sulfate, *Anomia simplex*, proteins


5. Adsorption properties of mussel based peptide sequences

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Mussels have the capability to stick on nearly every kind of materials like wood, stones, metals and plastics like PTFE. The composition of this protein based adhesive is well known currently. But the knowledge about the molecular interactions of the proteins and their amino acids, responsible for the remarkable adhesion properties, with the different surfaces which are responsible for the
remarkable adhesion properties is limited. This knowledge is essential for creating new artificial bio-inspired adhesives with comparable characteristics or well adjusted adhesive properties.

Different peptide sequences derived from Mefp-1 were synthesized by solid phase peptide synthesis for the evaluation of the influence of the “key” amino acid DOPA, its oxidation state and the length of peptide chains. The quartz crystal microbalance (QCM) technique is one of the methods of choice for the determination of substrate/peptide interactions. The investigations are supported by calculations of dissolved and adsorbed peptide structures using the conformers sampling [1]. Additionally, the tensile strengths of peptide glued tooth fragments were determined.

Keywords: mussel, adhesive, molecular modelling, QCM


6. In situ hybridization screening for genes of the duo-gland adhesive system of the flatworm Macrostomum lignano

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The free-living flatworm Macrostomum lignano possesses an elaborate adhesion-releasing system that enables the animals to adhere and release rapidly from the substrate (Tyler 1976). Our goal is to identify adhesion- and release-related molecules of the duo gland system. We recently initiated in situ hybridization (ISH) screening using probes selected from a tail-specific transcriptome. Bioinformatic analyses revealed approximately 400 transcripts with high expression in the tail region. From this gene list we selected 160 candidate genes based on the following criteria (1) a fold change of >=8 within the tail region compared to the region anterior to the ovary, (2) at least 100 mapped reads in the tail region, (3) less than 50 reads in the other regions, (4) BLAST results in the NCBI database, (5) a length of more than 200 bp. We report on an optimized whole mount ISH protocol that allows us to process 24 probes in parallel. Briefly, worms are kept in baskets, which are mounted in a modified 24-well plate cover.
To change the medium the cover with the baskets simply has to be transferred to a new, prefilled 24-well plate. In a preliminary screen we studied the expression patterns of 48 genes. We found staining of prostate glands, cells of the female antrum, gut-specific glands, and five candidate genes most likely involved in adhesion and release of *M. lignano*. We aim to analyse the expression patterns of the full complement of tail specific genes to identify the proteins involved in *M. lignano* adhesion and release.

**Keywords:** flatworm, screening, hybridization, duogland

**References:**

### 7. From sand tube to test-tube: characterization, production and testing of an adhesive protein from a marine worm

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Sabellariids are tube-dwelling marine polychaetes which live in the intertidal zone. They are commonly called honeycomb worms or sandcastle worms because they are gregarious and the tubes of all individuals are closely imbricated to form large reef-like mounds. To build their tube, they collect sand grains or mollusk shell fragments in their surroundings, dab them with spots of cement, and assemble them into a rigid composite tube. In *Sabellaria alveolata*, a common species along European coasts, the cement is produced by large unicellular glands housed in the anterior part of the animal and which open at the level of a specialized building organ made up of two lobes located near the mouth. Four of the adhesive proteins constituting the cement were identified based on their sequence similarity with proteins of a phylogenetically related species, *Phragmatopoma californica* [1]. Among them, the protein Sa-1 has a mass of 22 kDa and is rich in glycine, tyrosine and lysine residues, making it a positively-charged protein. It shows a repeated peptide motif whose consensus sequence is KGAYGAKGLGYGNKAGYGAYG. After synthesis of the coding genes, the adhesive protein Sa-1 was produced in *Escherichia coli* either in its full form or in
constructed multimeric form based on the repetitive pattern of its sequence. Sa-1 full forms has been successfully produced and purified at around 50 mg of pure protein per liter of culture while a yield of 10 mg/l was obtained for the multimeric form. As histochemical tests suggest that tyrosine residues in Sa-1 are presumably modified into DOPA, the recombinant proteins were enzymatically modified following purification by using mushroom tyrosinase [2]. Finally, the adsorptive and adhesive properties of the synthesized recombinant proteins were studied before and after enzymatic modification. The adsorption of proteins to glass was investigated by simple staining of deposited protein films. Adhesive properties were evaluated in lap-shear tests using both glass and steel as substrata. The mussel protein Mefp-3 and BSA were used as controls.

Keywords: Tubeworm, recombinant protein, dopa, lap-shear adhesion test


8. Characterization of the protein fraction of the temporary adhesive secreted by the tube feet of the sea star Asterias rubens

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Sea stars are able to make firm but temporary attachments to various substrata owing to secretions released by their tube feet. After tube foot detachment, the adhesive secretions remain on the substratum as a footprint [1]. Proteins presumably play a key role in sea star adhesion, as evidenced by the removal of footprints from surfaces after a treatment with trypsin [2,3]. Most of the proteins constituting the adhesive footprints were solubilised using denaturing and reducing buffers [4]. After SDS-PAGE analysis, these proteins separated into about 25 bands which range from 25 to 450 kDa in apparent molecular weight. Using mass spectrometry and homology-database search, it was shown that several of these proteins are known intracellular proteins, presumably resulting from a contamination of footprint material with tube foot epidermal cells. However, 11 protein bands, comprising the most abundant proteins, were not identified and might correspond to novel adhesive proteins. They were named “Sea star footprint proteins” (Sfps). Tandem mass spectrometry analysis of these protein
bands yielded 43 de novo-generated peptide sequences. Most of them were shared by several –if not all- Sfps. Polyclonal antibodies were raised against one of these peptides (HEASGEYYR from Sfp-115) and were used in immunoblotting. They specifically labelled Sfp-115 and other bands with lower apparent molecular weights [4]. Degenerate oligonucleotide primers designed on the basis of this peptide sequence permitted the amplification of a sequence of about 12 kb, comprising the 3’ end of the cDNA encoding for the protein. Experiments are currently in progress in order to obtain the 5’ end sequence of the cDNA. The translated sequence comprises about 3900 amino acids, with a calculated molecular weight of 437 kDa, largely higher than the apparent molecular weight of 115 kDa of the protein Sfp-115. Twenty three of the 43 peptides obtained with mass spectrometry were recovered in this sequence. Interestingly, only 13 of them were detected in the protein band Sfp-115, the others coming from the other Sfps. The different results suggest that all Sfps might be the products of the degradation of a very big protein or of an alternative processing of a unique mRNA. The protein sequence obtained is characterized by the presence of specific domains found in proteins with an adhesion role (especially to other proteins).

Keywords: Sea stars, adhesive secretions, proteins, mass spectrometry


9. Mapping sea urchins tube feet proteome – a unique hydraulic mechano-
sensory adhesive organ

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Many marine organisms secrete adhesives for substrate attachment that to be effective require functional assembly underwater and effective displacement of water, ions, and weakly bound polyions that are ubiquitous in seawater. Therefore, understanding the characteristics of these protein/carbohydrate-based marine adhesives is imperative not only to decipher marine attachment strategies but also to accelerate the development of new biomimetic underwater adhesives.
The present study aims at mapping the proteome of sea urchins specialized adhesive organs, the so-called oral tube feet, using a combination of two complementary techniques for protein separation (1DE-nanoLC and 2DE) followed by analysis with MALDI-TOF/TOF MS and protein identification using different databases and search algorithms. This strategy resulted in the identification of 315 non-redundant proteins from a total of 560 identified proteins (216 proteins by 1DE nano-LC, 76 proteins by 2DE and 23 proteins common to the two techniques), constituting to our knowledge, the first comprehensive list of sea urchin tube feet proteins. Given the known importance of phosphorylation and glycosylation in marine adhesion, the above results were complemented by profiling the 2DE proteome with specific fluorescent stains resulting in the identification of 66 non-redundant proteins from a total of 77 identified spots, of which 43 were exclusively phosphorylated, 16 were exclusively glycosylated and 34 had both PTMs. Although highly specialized for locomotion and attachment, oral tube feet discs are histologically complex and therefore a considerable fraction of the identified proteins in the total proteome are involved either in major cellular processes (35%) or cellular metabolism (23%). However, many proteins implicated in different processes such as development and regeneration (16%), nerve-related events (12%), immunological response (9%), muscle-related events (4%) and sensory perception (3%) were also found. Most importantly, putative adhesive and de-adhesive proteins have been identified, that although requiring further confirmation, constitute a step forward in the quest to decipher sea urchins temporary adhesion.

Keywords: sea urchin, tube feet, proteome, glycosylation, phosphorylation, adhesive proteins

10. Cell compatibility to salamander glue

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Once stressed by a predator, the red-legged salamander *Plethodon shermani* secretes a milky viscous liquid from its dorsal and ventral skin. The secretion is extremely adhesive and hardens within seconds when exposed to air. As a first step to characterise this glue as a potential system for clinical application, we
analysed the cell compatibility of the cured glue and the behaviour of the different cell types on it.
In order to harvest the glue, we stressed one salamander by touching it with a forceps and smeared the glue on a plastic foil (Aclarfilm). Afterwards the foils with the glue were seeded with two different cell lines (BRL-3A, smooth muscle cells) and primary chondrocytes. The cells were cultivated at 37°C and 5% CO2 in their standard media. Cells were also seeded on the pure Alcarfilm for control. After 2 and 7 days cells were treated with the LIVE/DEAD® Cell Viability Assays (Company Invitrogen No. L-3224) and analysed under the convocal laser scanning microscope. Afterwards the samples were fixed in 2.5% glutaraldehyde (in sodium cacodylate pH 7.2, 0.1 M), dehydrated and dried and examined in the scanning electron microscope.
The results showed clearly that the glue of *Plethodon shermani* is not toxic and that all cell types adhered to the salamander glue. However, slight different behaviour and cell shape on the glue in comparison to the pure Alcarfilm were visible, especially after 7 days. Future studies will focus on the glue composition by proteomic and molecular analyses.

**Keywords:** glue, salamander, clinics, biomimetic, cell culture, cytotoxicity

**References:** Janek von Byern, Ursula Dicke, Egon Heiss, Ingo Grunwald, Stanislav Gorb, Yannick Staedler & Norbert Cyran (2012) prepared for Zoology

### 11. Functional morphology and biomechanics of permanent attachment in climbing plants

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In the struggle for light climbing plants evolved various strategies to permanently attach themselves to vertical supporting structures like tree trunks or rocks. The most common climbing mechanisms are twining stems, coiling tendrils and adhesive organs. In plants with adhesive organs two attachment strategies are found: tendrils with adhesive pads or adventitious roots. In both systems efficient attachment is due to a combination of structural form-closure on the micrometre scale and/or usage of organic glue allowing the plants to climb flat and smooth supporting structures. The two types of adhesive organs evolved several times
independently in various plant families and thus might be evolutionary advantageous.

Based on our previous work on the attachment mode of *Parthenocissus tricuspidata* [1,2] and *Hedera helix* [3,4] we currently study the anatomy, morphology and biomechanics of attachment structures in other species of self-clinging plants. By comparing the structural, chemical and mechanical properties of the adhesive organs we aim to identify the underlying functional principles. Current results are presented.

Keywords: adhesive pad, adventitious root, vine, self-clinging plants


12. Stress analysis and study of the adhesion mechanism of permanent attachment pads of *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch

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Descriptions of permanent attachment systems of climbing plants can be found in literature since Darwin’s essay of climbing plants in 1865. Darwin described different kinds of climbing strategies and divided them into four classes: twining plants, leaf climbers, tendril bearers and hook and root climbers.

We are focusing on *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch, which belongs to the second class, the leaf climbers. An attachment pad is grown, which uses a combination of two different mechanisms to attach to the surface. One is the release of glue-like liquid that is secreted. The second is a mechanical connection by a tight form closure with the surface and subsequent shrinkage during the desiccation process. Since the upcoming of bionic and biomimetic science few mechanical tests have been accomplished on the different length scale, from nanoindentation to small scale tensile testing. Our previous studies of the structure and the mechanical behavior indicate a highly complex interaction between the different structural components of the pad. In order to get more insight into the inside of the pad we conducted μCT scans of adhesion pads grown
on different substrates. The structural analysis based on these scans was used to create finite element models to simulate the mechanical behavior and compare them to experimental results, e.g. micro tensile tests. The simulation challenges can be divided into a geometrical part, where optimization has taken place during evolution, and a material properties part to understand the inhomogeneous and anisotropic mechanical behavior of the lignified attachment structure. In this paper we will discuss our simulation results, the mechanical tests and the analysis of the µCT scans.

Keywords: Stress analysis, attachment pad, Parthenocissus tricuspidata, µCT, FE

13. Attachment ability of sawfly larvae to smooth surfaces

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Larvae of the sawfly Rhadinoceraea micans (Klug) (Hymenoptera, Tenthredinidae, Blennocampinae, Phymatocerini) adhere properly to the crystalline waxy surface of their host plant Iris pseudacorus L. (Iridaceae) by using three pairs of thoracic legs, seven pairs of abdominal prolegs, and a pygopod. On each leg and pygopod, smooth adhesive pads releasing a fluid were visualized using cryo-scanning electron microscopy. The attachment performance of living larvae was studied in centrifugal force experiments with smooth flat hydrophilic and hydrophobic glass surfaces. Such experiments with sawfly larvae or larvae of other insect species, having a similar stature, have not been carried out before. The body position on the centrifuge drum did significantly influence the friction force generation. Forces corresponded up to 19 times the larva body mass on normal glass and 14 on hydrophobized one. Although larvae generated significantly stronger forces on hydrophilic glass, they also attached properly to hydrophobic one.

Keywords: Insect, Attachment, Biomechanics, Cryo-SEM, Symphyta, Hymenoptera

14. The impact of biofilm on the attachment of mobile aquatic insects

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In comparison to terrestrial insects, which usually can attach directly to the substrate surface, for aquatic insects the situation is complicated due to the development of a surface of biofilm on the primary substrate. After just two hours of exposure in an aquatic environment, organic material, bacteria, and fungi form a primary biofilm on the surface of the substrate [1]. In comparison with the primary substrate, these biofilms are usually softer and change the surface structure as well as the surface chemistry. Roughness, material stiffness, wettability, surface chemistry and temperature can potentially have effects on adhesive strength [2]. Thus, the attachment conditions for benthic organisms can change significantly.

For many aquatic sessile animals (mostly marine), such as mussels, bryozoans, coelenterates or polychaetes, the effects of the biofilm on settlement and attachment have been previously investigated [3-10]. However, to the best of our knowledge, they neither have been subject of investigation in attachment studies on mobile freshwater organisms. Our recent investigations indicate that the biofilm strongly influences the ability of some aquatic insect larvae to attach to the substrate surface. So the reported ability of the running water mayfly Epeorus assimilis to attach to smooth surfaces [11,12] was no longer observed on sterile substrates [13]. The aim of the present study was to investigate the impact of the biofilm on the attachment of E. assimilis larvae.

We performed attachment experiments with live E. assimilis larvae in a flow channel using different substrates with defined surface roughness. Additionally, we measured attachment forces generated by dissected claws on the same substrates and mechanical properties of the biofilm. The experiments were performed on all substrates with and without biofilm.

Our results show that on substrates with smooth or slightly rough surfaces, where the claws hardly find surface irregularities to grasp on, the presence of the biofilm increases the friction force of claws significantly. Consequently, the larvae can endure higher flow velocities on these smooth substrates. The opposite effect takes place on rough substrates, where the attachment force of claws decreases in the presence of biofilm. Consequently, biofilm is an important impact factor on the ability on the larvae to attach to the substrates in various stream habitats.
Keywords: Biofilm, periphyton, surface texture, claw, attachment, functional morphology


15. Probing the effect of surface roughness on cockroach adhesive pads using microstructured substrates

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Many insects are able to adhere well to vertical and inverted smooth and rough surfaces: this is achieved via a conformable adhesive component (either a smooth, soft pad or a dense array of hairs) along with a thin film of adhesive fluid [1]. In nature, many plants have developed slippery surfaces as defense mechanisms which are able to prevent insects from climbing. These slippery surfaces often employ a high degree of surface roughness which may prevent the adhesive pad conforming fully to the surface [2]. The ability for the adhesive pad to conform to a surface is important in producing adhesive or shear force as it creates a large interface between the adhesive element and surface; reducing this interface will have the effect of reducing the adhesive force [3,4]. However, it is difficult to study or predict precisely how insect adhesive pads conform to naturally rough surfaces, as they typically consist of several length scales, where it is uncertain what the effect of each length scale is, and are opaque, preventing viewing of the pad/surface interface. To overcome these problems, we have tested cockroach adhesive pads on transparent rough surfaces with a single length scale.
Using standard photolithography techniques and a soft casting method, we produced a series of transparent epoxy films patterned with arrays of micrometre-sized pillars with height of 0.5 or 1.4 µm and separation varying from 3 to 22 µm. The interface between adhesive pad and surface was visualized using reflected light by imaging through the rear of the transparent substrate. Shear stress measurements on single pads were carried out on all substrates. Full contact was found for all arrays with 0.5 µm tall pillars, with little difference in shear stress observed. However, a transition from full to partial contact was observed for the densest array of 1.4 µm tall pillars. The transition from full to partial contact is consistent with a simple model based on indentation theory. A drop in the measured shear stress, calculated using the projected contact area, was measured at the transition from full to partial contact.

Our findings may be useful for understanding the mechanisms of insect adhesion on natural rough surfaces, and for designing surfaces which reduce insect adhesion.

Keywords: Insect adhesion, structured surfaces


16. Can the green crab *Carcinus maenas* be a biomimetic model for antifouling technologies in marine sensors?

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The continuous research on new antifouling methods to protect sensors, even for long time periods, in the marine environment, has led the scientific community to look for a source of inspiration from nature to find out new environmentally and economically acceptable methods to control the biofouling problem. The biomimetic approach, indeed, represents nowadays one of the most promising ways to find out an effective and eco-friendly alternative to the existing antifouling methodologies (Ralston & Swain 2011). In the marine environment every metazoan has sensors (mechano/chemo-sensors) and the maintenance of their performance is extremely important. Marine organisms use mechanical,
chemical, physical and behavioral techniques to protect themselves against fouling. In this study the decapod crustacean *Carcinus maenas* (green crab) was selected as model organism and its antifouling (anti-epibiosis) techniques, particularly regarding its sensors such as the eyes, were investigated. A behavioral study on the eye cleaning techniques in the green crab was performed by means of video recording with a high speed camera: four different eye cleaning behaviors were identified and characterized. Moreover the general eye morphology and all structures and the appendages involved in the cleaning techniques were observed by Atomic Force Microscope and Scanning Electron Microscope. The results of behavioral studies and morphological investigations on the eye of *C. maenas* revealed an unexpected potential link between the biomechanics of the eye cleaning process and the external anatomy of crab eyes. Scanning electron microscope investigation, indeed, pointed out the presence on specific areas of the eye stalk of outlet pores, probably belonging to tegumental glands. The pores position showed a strict relation with the trajectories and biomechanics of the identified cleaning behaviors. Furthermore the topography of the eye, investigated by Atomic Force Microscopy, pointed out the presence of features likely to be involved in preventing the attachment of unwanted organisms on the visual surface. The results of this study pointed out that *C. maenas* can be considered a valuable biomimetic model organism for antifouling strategies to protect marine sensors, and it is possible to envisage a potential chemical protection strategy to prevent the adhesion of organisms on the crab eye surface not so far recognized.

Keywords: biomimetic, antifouling, crustacean, AFM, SEM, cleaning behaviour


**17. Insights into the structure and function of tree frog adhesive toe pads obtained from cryo-SEM and atomic force microscopy**

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As neither cryo-SEM nor atomic force microscopy involve fixation, the problems of shrinkage and distortion that occur during tissue preparation are avoided. Indeed, the cryo-SEM is particularly appropriate as it enables the visualization of the fluid layer that is critical to a toe pad’s adhesive function. We examined two
frog species, the Malaysian flying frog, *Rhacophorus prominans* (Family Rhacophoridae) and White’s tree frog, *Litoria caerulea* (Family Hylidae). This enabled us (1) to compare pad surface nanostructures in two distantly related species, where toe pads can be presumed to have evolved independently (AFM); (2) to view cytoskeletal structures in pads fractured after freezing in liquid nitrogen (cryo-SEM); and (3) to view frozen frog footprints, thus gaining an impression of the relative thickness of the fluid layer under different parts of the pad.

Overall, we found extraordinary similarity between structures in the two species examined. Given their independent origins, this suggests that there is an optimal structure for a toe pad, which may have implications for biomimetics. The epithelium is multi-layered, the outer layer being shed at intervals. Such frequent replacement of the outer surface avoids problems of wear. The cells are mostly hexagonal, with deep channels between them. In our footprint studies, these channels were seen to be fluid-filled. The fluid extended all over the ventral surface of the pad, ending in a thicker layer around the pad perimeter. This is surprising as it would reduce the capillarity forces that are important for toe pad adhesion. An array of nanopillars covers the surface of these cells, each nanopillar having a concave top. The most prominent of the cytoskeletal structures are keratin fibrils that lie at an angle to the surface. They extend inwards from the nanopillars and are presumably important for strong adhesion. Fibrils lying at angles to the surface are also found in insect smooth adhesive pads.

**Keywords:** Cryo-SEM, atomic force microscopy, tree frog, adhesive toe pads, biomimetics, cytoskeletal structures


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**18. Attachment and detachment of the tree frog, *Litoria caerulea*, during walking and climbing**

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Tree frogs are capable of adhering to a variety of substrates using their toe pads. They accomplish this by wet adhesion, secreting a thin layer of fluid between their
pads and the substrate. This adaptation is extremely valuable in the natural world, playing a role in both static and dynamic situations. It also serves to highlight tree frogs as a model for the development of bioadhesives that are capable of functioning in wet conditions.

The adhesive abilities of these frogs have been widely studied, mostly with regards to the mechanism of adhesion utilized and to the morphology of their toe pads. However, less is known about the general mechanics of their locomotion, and the movement of the toe pads and limbs during attachment to and detachment from a substrate; more specifically, how rapidly these actions occur and in what manner they're accomplished. Understanding these aspects of their adhesion will not only add to the ‘catalogue’ of animals already studied in this way (such as geckos), but will also shed more light on the function of the toe pads during locomotion and thus aid in the manufacture of effective bio-mimics.

This presentation aims to show that: a.) attachment of the toe pads tends to take longer than their detachment, b.) the rates of attachment and detachment are independent of walking/climbing speed, and that c.) the ‘style’ of attachment is variable, whereas detachment generally occurs from the rear of the pad – irrespective of the mode of locomotion. Future work will explore variations in the magnitude and direction of ground-reaction forces between walking and climbing, and in particular the force required to detach the toe pads. These experiments, combined, will attempt to answer the question as to how these pads can generate large adhesive and friction forces when required, yet detach effortlessly.

Keywords: Frog, adhesion, peeling, locomotion


19. Evaluation of the adhesion properties of biomimetic polymer surfaces by atomic force microscopy

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1Laboratory for Chemistry of Novel Materials; 2Laboratory of Polymeric and Composite Materials; Center of Innovation and Research in Materials and Polymers (CIRMAP); 3Laboratory of Marine Biology - University of Mons (UMONS), Mons, Belgium
Interest in biomimetic materials, which aim to mimic the properties of natural materials, is growing. In particular, the adhesive properties of biological origin (mainly based on natural (co)polymers) are very interesting for applications in various fields such as adhesion in aqueous media.

Our research project focuses on the characterization of the morphologies and the adhesion properties of biomimetic copolymer thin films by Atomic Force Microscopy (AFM). We synthesized block copolymers containing a segment of Poly (dimethylsiloxane) (PDMS) and a segment of ionogenic poly-(2-dimethylamino) ethyl methacrylate (PDMAEMA) or polyacrylic acid (PAA) which can be easily protonated in aqueous media by changing the pH. These copolymers were synthesized by atom transfer radical polymerization (ATRP). The copolymer films were prepared by spin-coating which enables the fabrication of homogeneous thin films with controlled thickness. The AFM results show micro-phase separation for PDMS-b-PDMAEMA copolymers (in this case the solvent is a good solvent for both blocks) and micellar morphologies for PDMS-b-PAA copolymers when the solvent is selective for one of the blocks [1-3]. This is the result of the immiscibility existing between different blocks of the copolymers. The copolymers were also incorporated into a cross-linked PDMS matrix to prepare films producing adhesive properties in aqueous media. The morphologies and adhesive properties of their films before and after immersion in water were determined with AFM. The adhesive properties of the films were also tested by experiments with marine animals (mussels, sea stars).

The influence of positive and negative charges on the behavior and adhesion coatings was also investigated. For this purpose, we synthesized polyzwitterionic polymers such as PDMS-b-PDMAEMA (SO3-) which have both cationic and anionic charges on the same side group, thus creating permanent dipoles.

20. Surface modification with lipoic acid derivates for modulation of wettability and adhesion of macromolecules

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Lipoic acid derivates can be applied for surface modification due to attraction disulfide group to gold [1]. This contribution showed design, synthesis and surface characterization of new derivate after formation of monolayer at the surface. Cationic derivate can be used for switching between superoleophobic and superhydrophobic and hydrophilic can be vary by means of applied contra anion and surface topology. This study also demonstrates possibility to modulate adhesion of biomacromolecule by light responsive or zwitterionic character of the applied derivate. Controlled adhesion and release of DNA molecules on the surface is observed via quartz crystal microbalance and fluorescence techniques.

ACKNOWLEDGEMENT: This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the Grant VEGA No. 2/0152/10. This contribution is also the result of the project implementation: Centre for materials, layers and systems for applications and chemical processes under extreme conditions supported by the Research & Development Operational Programme funded by the ERDF.

Keywords: lipoic acid, switchable, superoleophobic


21. \textit{In-vitro} results on self-adhesive resin cements

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\textsuperscript{1}Raluca Ripan Institute of Research in Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania; \textsuperscript{2}Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy Timisoara, Romania; \textsuperscript{3}Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University of Cluj-Napoca, Romania

Objective: The aim of this study was to evaluate the push-out bond strength of two self-adhesive resin cements used for the cementation of glass fiber posts.
Materials and Methods: Twenty recently extracted non-carious human maxillary central incisors, with fully developed apices extracted for periodontal reason were selected for this study. After endodontic treatment, glass fiber posts (RelyX Fiber Post, Size 3, 3M ESPE) were cemented with two types of self-adhesive resin cements (RelyX U200, 3M ESPE, and Maxcem Elite, Kerr). Perpendicular to the post, four to six sections of 1 mm height were cut from each specimen using a diamond saw (Isomet 1000, Buehler) starting 1 mm coronal from the tip of the post. Each section was tested on push out bond strength with the testing machine (0.5 mm/min, Lloyd Instruments Ltd, Fareham Hants, UK). The retentive strength of the post fragment (MPa) was calculated by dividing the load at failure (N) by the interfacial area of the post segment. Failure modes were evaluated by a single operator under a stereomicroscope (Stemi 2000-C; ZEISS, Jena, Germany) at 40x magnification and SEM.

Results: Push out bond strength of RelyX U200 (8.3±2.7) was statistically higher than that of the Maxcem Elite (6.4±3.9). Failure mode was predominantly at resin cement/dentin interface. The results recorded were statistically different when was evaluated with one-way analysis of variance and Tukey test (p<0.05).

Conclusions: Push out bond strength was influenced by cement type and varies upon different regions of the root canal. Adhesive interface between resin cement and root dentin was the most fragile region.

ACKNOWLEDGEMENTS: The study was done within the frame of the COST Action TD0906 Biological adhesives from biology to biomimetics. One of the authors (G.F.) thanks the Babes-Bolyai University of Cluj-Napoca for the financial support through the POSDRU/89/1.5/S/60189 Grant.

Keywords: fiber post, push-out bond strength, self-adhesive resin cement

22. Surface structure of three bioadhesive systems with potential applications in dentistry. AFM investigations

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Introduction: Development of resin-based adhesive biomaterials that can adhere to hard dental tissues and that can be applied using minimally invasive techniques is a continuous interest of researchers in the field. Objective: The aim of the
The present work was to investigate the morphology and to evaluate the degradation of the surface of three new experimental resin-based filled bioadhesive systems with potential applications in dentistry, exposed to artificial saliva. Materials and methods: 1. Obtaining of bioadhesive systems: Three experimental filled bioadhesive systems have been formulated using a resin based on new synthesized Bis-GMA-type oligomers and three inorganic fillers, an aluminum-fluorosilicate glass, a new synthesized nano hydroxyapatite and a commercial hydroxyapatite (Sigma). The adhesives were stored in artificial saliva for 30 days. 2. The investigation of the surface morphology of the experimental filled bioadhesives was done using the scanning probe microscope, AFM, JEOL 4210 equipment operating in the intermittent contact, tapping mode. The bioadhesives were examined after 1, 7 and 30 days of storage in artificial saliva. The surface roughness, described by the rms (root mean square) value was calculated directly from the AFM observation by the processing of topographical AFM images. Results and discussion: After 1 day of storage, the bioadhesive containing the synthesized nano hydroxyapatite (BA1) features the most uniform and compact surface having the minimum sample height, respectively the lowest roughness (133 nm), followed by the bioadhesive containing the commercial hydroxyapatite (BA2) characterized by a roughness of 217 nm and finally by the bioadhesive filled with aluminum-fluorosilicate glass (BA3) which presented the highest surface roughness of 240 nm. After 7 days and respectively 30 days of storage in artificial saliva, the surface roughness was 129 nm and respectively 103 nm in the case of BA1 adhesive, 208 nm and respectively 125 nm for BA2 adhesive, 177 nm and respectively 262 nm for BA3 adhesive. Conclusion: From the viewpoint of the surface uniformity, we can conclude that the smoothest surface results from the bioadhesive based on synthesized nano hydroxyapatite, followed by adhesive system based on commercial hydroxyapatite, and the highest roughness being observed for the adhesive based on glass filler particles. All three bioadhesive systems present rather compact structures and are relatively stable to artificial saliva for 30 days. The present study proves that AFM observations make possible the analysis of the surface structure for filled bioadhesives as well as the characterization of their stability in a biological medium (e.g., artificial saliva).

Keywords: bioadhesives, dentistry, nano hydroxyapatite, AFM technique

23. The use of cell outer membrane mimetic surface in order to decrease adhesion of blood cells on the surface of artificial heart

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Warsaw University of Technology, Faculty of Chemical and Process Engineering, Department of Biotechnology and Bioprocess Engineering, Laboratory of Biomedical Engineering

Polyurethanes and other polymers that are nowadays in use in construction of artificial cardiovascular implants need to be modified to meet current strict requirements in terms of biocompatibility and especially compatibility with blood. This is caused by the phenomenon of clotting of blood on the unnatural surfaces which occurs during a complex process called blood coagulation cascade. It proceeds in the same way when naturally triggered by a damage in a blood vessel as when started by other impropriety of a surface contacting with blood [1]. Thus, every artificial device that contacts with blood in the body of patient poses a threat of spontaneous formation of clots [2]. Natural blood vessels, unlike the artificial ones, have their own natural protection against spontaneous formation of blood clots. This protection is provided by endothelium cells, which are the cells that cover the inner surfaces of all blood vessels. Endothelium cells contact each other very closely creating a very homogenous layer of a clot-protecting coating which prevents from adhesion of platelets, clotting factors and other small and large molecules on the walls of blood vessels [3].

Numerous attempts have already been undertaken to modify surfaces of existing materials in order to provide necessary protection against clot forming agents adhesion, very often by coating the materials with bioactive substances. In the last few years many of these studies involved the use of phospholipids in order to create coatings in form of cell outer membrane mimetic surfaces that would be acting like cell membranes of living organisms [4,5].

The aim of this study was to develop a simple method of preparation of a phospholipid coating on a surface of polyurethane. It has been achieved by the use of dip-coating method in which materials are immersed in a modifying solution in order to obtain coatings on their surfaces. The efficiency of this method has been confirmed by chemical analysis of the materials’ surface layers with the use of FTIR-ATR technique. Evaluation of the wettability of the obtained materials showed very significant increase in hydrophilicity, which confirms the ability of phospholipid particles present in the coating to obtain the confirmation similar to these present in natural cell membranes. SEM analysis showed a significant decrease in the number of blood cells adhering to the surface of the materials. The analysis of hemocompatibility and cytotoxicity proved that
obtained materials are safe, clot resistant, and therefore potentially useful in cardiovascular implants construction.

Keywords: phosphatidylcholine, polyurethane, cell outer membrane mimetic surface, artificial heart


24. Geometry-controlled adhesion: testing hexagonal surface pattern

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Following studies of hairy attachment pads evolved in different animal groups, an interesting correlation between the size of attachment hairs and animal weight was found: the heavier the representatives of particular animal group, the smaller and more densely packed the microstructures on the adhesive pads. Based on this finding, it was hypothesized that splitting up the large contact into finer subcontacts of the same total contact area increases adhesion on flat smooth substrate. To examine this hypothesis, we experimentally tested hexagonally-patterned polymer samples having constant contact area and different number of subcontacts. Our measurements clearly demonstrated that, in flat-punch-patterned conformal contact, the pull-off force remains the same, even when the number of subcontacts increases by two orders of magnitude. Our finding suggests that the contact splitting principle on the flat substrate can only work in thin-film-based contacts, which are indeed employed in most biological temporary attachment systems.

Keywords: Biomimetics; surface patterning; contact mechanics; scaling; fibrillar attachment
25. Biodegradable adhesives based on cross-linkable (meth)acrylates

Ralf Wyrwa¹, Albrecht Berg¹, Ebru Toksoy Oner² & Matthias Schnabelrauch¹

¹ INNOVENT e. V., Biomaterials Department, Jena, Germany; ² Marmara University, Department of Bioengineering, Istanbul, Turkey

For many years (meth)acrylates have been successfully used in medical applications like treatment of bone defects or dental fillings. Recently cross-linkable materials based on (meth)acrylates have been intensively studied for their use in tissue engineering applications as well as bone and tissue adhesives. Especially concerning adhesives, there are urgent clinical needs in trauma and reconstructive surgery for bonding of soft tissue, internal organs, and bone fragments as well as primary fixation of implants. Due to high adhesion strengths synthetic (meth)acrylate based adhesives are well suited for these applications but often their innate bioinert behaviour impairs biodegradation and replacement by body’s own tissue. In this context a major focus of our research is the development of biodegradable adhesive systems based on synthetic monomers and natural macromers based on biopolymer like polysaccharides, glycosaminoglycans and gelatin. After thermally or photochemically induced cross-linking of those biopolymer-based adhesives, the resulting materials should exhibit hydrolytic or enzymatic degradability forming low-molecular fragments which could be further metabolized by cells of surrounding tissue or completely excreted from the body.

Introducing new biopolymers like levan into the pool of (meth)acrylated adhesive components will increase the possibilities to adapt the special properties of adhesives to the requirements of specific medical indications. Properties of selected (meth)acrylated adhesive systems will be discussed emphasizing the clinical applicability of (meth)acrylated biopolymer adhesives.

The European Union Seventh Framework Programme under grant agreement no. NMP4-SL-2009-229292 is gratefully acknowledged for financial support.

Keywords: biopolymer, (meth)acrylate, levan, adhesive
26. Toward cost-effective fabrication of hierarchical gecko-mimicking structures using a new hot pulling and embossing technique

H. Hölscher, M. Röhrig, G. Etienne, F. Oulhadj, M. Schneider & M. Worgull

Institute of Microstructure Technology, Karlsruhe Institute of Technology (KIT)

We present hierarchical gecko-mimicking dry adhesives fabricated by the combination of hot embossing and our novel hot pulling technology. The hot pulling process enables the cost-effective large-area fabrication of delicate polymer fibrils with highest aspect ratios and diameters in the nanometer range. Combining hot pulling with hierarchical hot embossing, we fabricate threefold hierarchical fibrillar structures in the micro- and nanometer range. The fabricated structures are therefore very compliant and achieve a high real contact area even to rough substrates. This causes high adhesion, originated by van der Waals forces. Analyzing the adhesion by atomic force microscopy we verify the high potential of these structures. In addition to classical thermoplastics, biodegradable polymers are suitable for this fabrication process as well. Consequently, the presented hot pulling and embossing process represents a way towards mass production of a commercial gecko-tape suitable for a broad range of applications.

Keywords: Dry adhesives, hot embossing, hot pulling, Hierarchical Gecko-type structures


27. Learning twice with nature to produce new polystyrene-based biomimetic films

Ana I. Neto¹,², Jonathan J. Wilker³ & João F. Mano¹,²

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Nature has inspired scientists achievements and has led to effective materials, structures, tools, mechanisms, processes, methods, and many other benefits. Materials found in nature combine many peculiar properties that have been
inspired the development of novel biomimetic materials, such as micro/nanostructures surfaces with extreme wettability [1-3] and marine-inspired adhesives [4]. This work intends to developed products combining this two particular characteristics.

Superhydrophobic polystyrene surfaces were produced inspired by the water repellent behaviour of surfaces of natural coatings of several animals or plants, such as Lotus leave. At the same time, a random copolymer with a 3,4 dihydroxystyrene-co-styrene backbone was used to take the place of the protein polyamide chain of DOPA (3,4-dihydroxyphenylalanine) [5]. Such polymer, mimics the catechol side chain of DOPA (an amino acid that is believed to be responsible for both adhesive and crosslinking characteristics of mussels adhesive proteins) and is similar to styrene, with only two hydroxyls added.

The styrene-catechol copolymers also display enhanced adhesion upon cross-linking and comparable to cyanoacrylate “super” glues and can bond to nearly any surface, including superhydrophobic surfaces.

The main objective of this work is to combine these two synthetic polystyrene-based materials to create asymmetric films exhibiting one superhydrophobic side and the other face with adhesive features. These devices could be glued to a variety of substrates and shaped into tubular objects with superhydrophobic inner regions that might be used as artificial blood vessels or in devices for fluid transport.

Keywords: Biomimetic, bio-inspired adhesives, superhydrophobic surfaces, polystyrene

Over the last few years, biopolymers have attracted a huge interest due their usefulness in diverse applications in biomedical and pharmaceutical industries. Polysaccharides-based biomaterials are associated to a large range of characteristics and properties, able to be used on tissue regeneration, drug delivery devices and gel entrapment systems for the immobilization of cells. Marine mussels, *Mytilus edulis*, generate a natural impressive adhesive material that can bond to nearly any surface. In the case of secreted adhesives such as those employed by marine organisms, biomimetic efforts are only possible when there is basic understanding of the “key” macromolecular components and their compositions. The adhesive proteins of mussels contain high concentration of catechol and amine functional groups allowing the adhesion to most of the organic and inorganic surfaces. However the role of 3,4-dihydroxy-L-phenylalanine (DOPA) in mussel adhesive proteins is not fully clear. It is known that the oxidized DOPA residues play important function on cross-linking reactions leading to solidification of the secreted liquid protein adhesive [1]. The low molecular weight of catecholamine mimics the proteins, such as dopamine at alkaline pH, to form adherent polymer coatings on a large variety of substrates, suggesting that synthetic polymers with catechol and amine functionalities may be used in the fabrication of devices with adhesive properties. Electrostatic self-assembly has become an alternative technique to modify biopolymers surface properties, due to the ease of the procedure and to the large amount of materials that may be employed [2]. Self-assembled nano-multilayer’s using layer-by-Layer (LbL) methodology have been produced using marine-origin polymers in our research group, being useful to a large variety of biomedical applications including tissue engineering, drug release, biomimetic coatings and smart surfaces to control adhesion of proteins/peptides and cells attachment [3-6]. Inspired by the structure and properties of mussel adhesive proteins, new LbL films based on chitosan (CHT) and DOPA modified hyaluronic acid (HA) were successfully developed. We hypothesize that the conjugate HA-DOPA can form multilayers with CHT and produce surfaces with distinct properties with respect with the conventional CHT/HA ones, including improved adhesive properties and an enhanced biological performance. The preparation of multilayer’s using this LbL is achieved by alternatively dipping a substrate into solutions of polyelectrolytes of opposite charge. By doing that deposition procedure for several times it is possible to process multilayer film in a controlled way to produce strong adhesive bandage for wounds skin. The sequential build-up of the multilayered films permits to control the materials that are deposited along the thickness of the film. In this work we use this possibility to create asymmetric films, in which, for example, one of the face will be more rich in DOPA-containing HA and the other
face will be more rich in non-modified HA. This could permit to produce films with adhesive properties in just one of the sides.

Keywords: Adhesion, Layer-by-Layer, Dopamine, Marine mussels


29. Increasing the functionality of DOPA and dopamine functionalized materials by substitution of the catechol ring

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Max-Planck-Institut für Polymerforschung, Mainz, Germany

Mussel adhesive proteins possess a high content of 3,4-dihydroxyphenylalanine (DOPA) residues in their structure. The catechol units, with a versatile chemical reactivity tuned by sea water triggers (pH, salt, ions), undergo crosslinking and chelation reactions and generate a material with high interfacial and cohesive mechanical strength and durability. The introduction of additional substituents to the catechol unit allows extension of the properties of DOPA-functionalized materials far away from adhesiveness. We demonstrate how Cl-substitution and NO2-substitution of the catechol ring confer antimicrobial and light-triggered detachment properties.

Keywords: DOPA and dopamine, attach and detach, photosensitive


30. Enhancing friction and grip of suturing threads to tissue

Cristina Serrano, Juan P. Fernández-García & Aránzazu del Campo
Max-Planck-Institut für Polymerforschung, Mainz, Germany
Hairy surface designs in the attachment pads of flies, ants and geckos allow these animals to reversibly adhere to almost any surface. Bioinspired analogues of these systems have been prepared and studied over the last 10 years. Different patterning techniques based on methods from the microelectronic industry (mainly lithography and dry etching combined with embossing and soft moulding) and flat substrates have been used for these purpose. We present a new patterning method based on plasma etching of polymer materials of any geometry to create gecko-like nanopatterns. Pattern generation is based on differential etching of crystalline domains in the material with a morphology that is previously determined by the mechanical and thermal pretreatment of the sample. We apply this method to the generation of fibrillar and lamellae nanotopographies onto textile fibers like suturing threads. The nanotopography is expected to enhance fixation to tissue without damaging it.

Keywords: plasma etching, surface nanopatterns, suturing threads

31. Light tunable materials for controlled adhesion and release biomacromolecules

Patrik Sobolčiak¹, Miroslav Mrlík²,³, Vladimír Pavlínek²,³ Igor Lacík¹, Peter Kasák¹

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Light switchable materials can be utilized in many applications such as biosensing, drug delivery, and tissue engineering. [1]
In this contribution presents newly prepared light switchable material which can be switch between two forms. One form is polycationic stage owning sticky, highly adhesive and antibacterial properties and second form is zwitterionic stage which is nonadhesive and biocompatible. Switch can be carried out by means of photolysis with UV light at 365 nm.
This study also demonstrates that polycationic form can condense anionic DNA molecules [2] and release can be tunable with target photolysis. Potential of condense and release DNA were confirmed by electrophoresis, fluorescence and surface plasmon resonance techniques.
Rheological studies exhibit tunability of mucoadhesive properties of this material by means of light.
ACKNOWLEDGEMENT: This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the Grant VEGA No. 2/0152/10 and with support of Operational Program Research and Development for Innovations co-funded by the European Regional Development Fund (ERDF) and national budget of Czech Republic, within the framework of project Centre of Polymer Systems (reg. number: CZ.1.05/2.1.00/03.0111).

Keywords: photolysis, switchable, gene delivery, mucoadhesive

References:

32. Mushroom-shaped dry adhesives

Sabine Akerboom, Mark van Turnhout, Johan van Leeuwen, Marleen Kamperman

Physical Chemistry and Colloid Science, Wageningen University and Research Center, The Netherlands

A feature that is often observed for animals that can walk on the ceiling (e.g. insects, spiders, geckos) is an array of pillars with caps (so-called mushroom-shaped pillars). We developed a method to make mushroom-shaped structures using self-assembled crystalline layers of polystyrene particles (900 nm in diameter) as template. Finite element analysis of our structures shows that pillars without caps, or with small caps are unfavourable and that the maximum stress at the surface switches from the edge to the center of the mushroom-shaped pillar when the cap size increases.
### LIST OF PARTICIPANTS (by alphabetic order)

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