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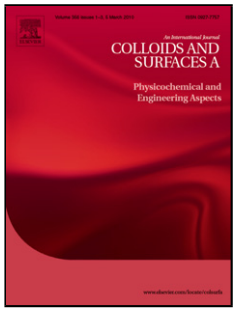

Colloids-at-surfaces: physicochemical approaches for facilitating cell adhesion on hybrid hydrogels

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Colloids-at-surfaces: physicochemical approaches for facilitating cell adhesion on hybrid hydrogels

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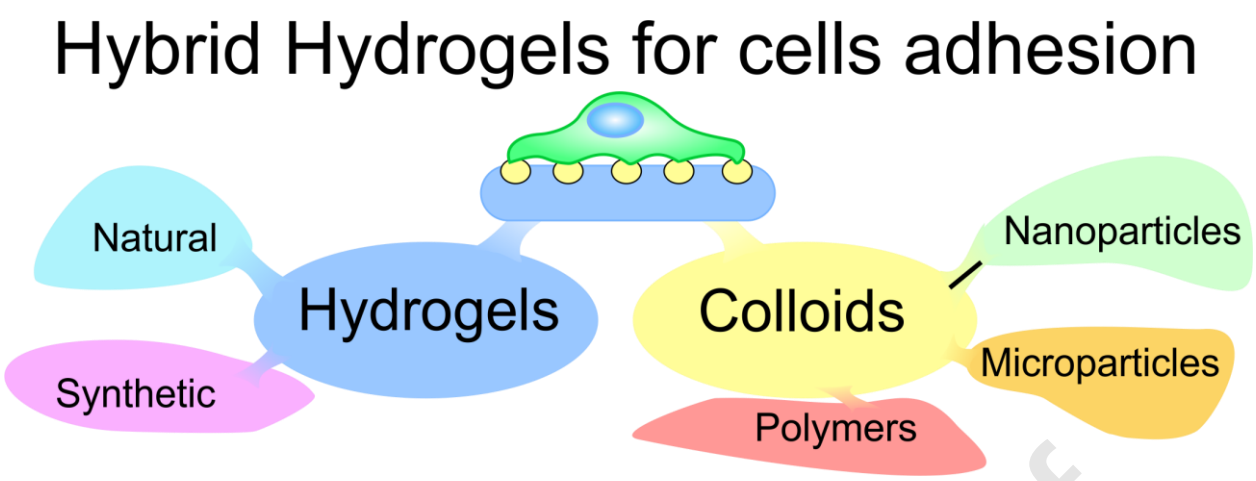
Graphical abstract



Abstract. Implementation of an effective focal cell adhesion represents a significant challenge because it requires to develop appropriate materials and processes together with assuring that cells would interact with it effectively. Various coatings are under development in the area of biomaterials including hydrogels and polymeric surfaces. Here, we analyse modification of the coatings by colloidal nano- and micro-particles, which effectively modify the surface of soft hydrogel materials, enhance and allow for adjustment of mechanical properties, and enable molecule release capabilities. A classification of such hybrid coatings is presented, where natural and synthetic polymeric coatings are overviewed. These organic coatings are modified by inorganic micro- and nano- particles. Various approaches to the design of such hybrid coatings are overviewed, while additional functionalities such as release of encapsulated biomolecules and enhancement of mechanical properties are highlighted. The developments in this area target effective cell growth, which is shown to be enhanced by the addition of colloidal particles.

Keywords: hydrogels, mineralisation, particles, focal adhesion, polymers.

Introduction

A substantially high number of polymers can bind to form a three-dimensional network, which can contain a large amount of water, therefore, forming a hydrogel of natural or synthetic origin.[1] Most of them are biodegradable and biocompatible, which allows them to be used for biomedical purposes.[2] Thus, naturally derived hydrogels that are similar to native extracellular matrices (ECMs), with a fine 3D network, high water content, good biocompatibility, and versatile fabrication methods, have emerged as promising matrices for the fabrication of biomaterials.[3] Nevertheless, they may have poor mechanical properties and lack binding sites for cell adhesion proteins, which leads to a lack of cell adhesion on their surface.[4] But the surface properties of hydrogels can be changed by modification with colloidal polymers, nano and microparticles.[5] As shown by numerous studies, such a modification can change the macro and micromechanical properties,[6,7] give the cells the required number of binding sites,[8] which will ensure a strong connection of cells with the surface. Surface modification can improve not only cell adhesion, but also provide additional functionalisation, for example, the second component may contain drug molecules.[9] It is also possible to control not only adhesion but also give the cells the desired shape,[8] and direct their growth in the right direction.[9]

In this regard, we need to classify sub-areas and components of hybrid hydrogels encompassing a modification of hydrogels by colloidal, nano and microparticles, and polymer complexes.

Overall, this can be structurally classified as follows:

(1) pure hydrogels, which can be sub-divided:

(a) natural hydrogels (alginate,[10] chitosan,[11] collagen,[12] gellan gum,[13] hyaluronic acid,[14] gelatin,[15] natural peptides,[16] *etc*);

(b) synthetic hydrogels (Poly(2-hydroxyethyl methacrylate),[17] polyethylene glycol,[18] poly(acrylic acid),[19] poly(methacrylic acid)[20], synthetic peptides,[16] *etc*).

(2) Colloids:

(a) nanoparticles (AuNPs,[21] SiNPs,[22] FeNPs,[23] BGNPs[24], *etc*);

(b) microparticles (CaCO3,[25] SiO2,[26] Ca10(PO4)6(OH)2)[27], *etc*) ;

(c) polymers (Layer-by-Layer,[28] Brushes,[29], *etc*).

This structure is summarised in **Figure 1** with additional details. Not surprising that colloidal science, which deals with polymers, inorganic nanoparticles and microparticles, etc. and their modification, has become a distinct discipline.

Hybrid hydrogels

Polymer hydrogels are a large class of soft materials consisting of hydrophilic molecules cross-linked chemically or physically in a network.[1] Due to the aquatic environment and imitation of biological tissues, hydrogels are widely used for medical purposes: from creating eye lenses to transplanting cells into damaged tissues and the remote delivery of drugs in the body.[30] Hydrogels, both natural and synthetic, can be formed by covalent bonds, physical cohesion forces between the polymer segments such as ionic bonding, hydrogen bonding, van der Waals forces, and hydrophobic interactions.[31] In the last decades, hydrogels have received growing attention because of their easily tunable properties, making them broadly applicable to many promising and emerging biomedical applications.[3] But often the surface of the hydrogel has a drawback - poor cell adhesion.[32] This problem can arise both because of poor mechanical properties, the chemical structure of hydrogel molecules and the absence of binding sites.[33] In this case, hydrogels require a qualitative modification, which would allow a hydrogel with poor cell adhesion to become a good bio-interface.[34] The second colloidal component acts as such a

modification of the hydrogel surface.[29] These can be polymers applied by the Layer By layer (LBL) system,[20,35] nanoparticles that are adsorbed onto the surface,[36] and microparticles grown on the surface of the hydrogel as a result of biomineralisation.[37] The second plus of surface modification of hydrogels is the possibility of additional surface functionalisation by loading bioactive molecules.[9]

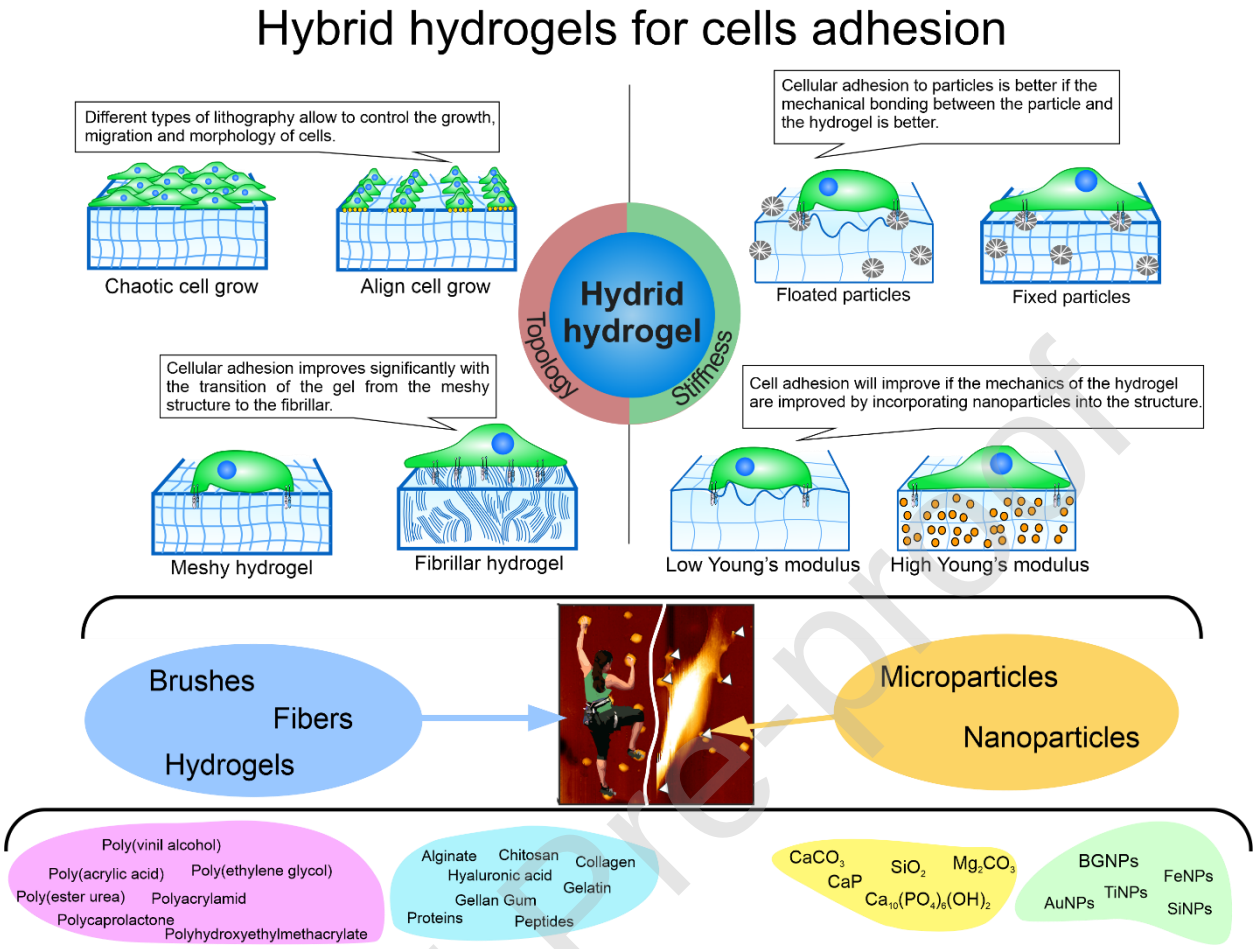
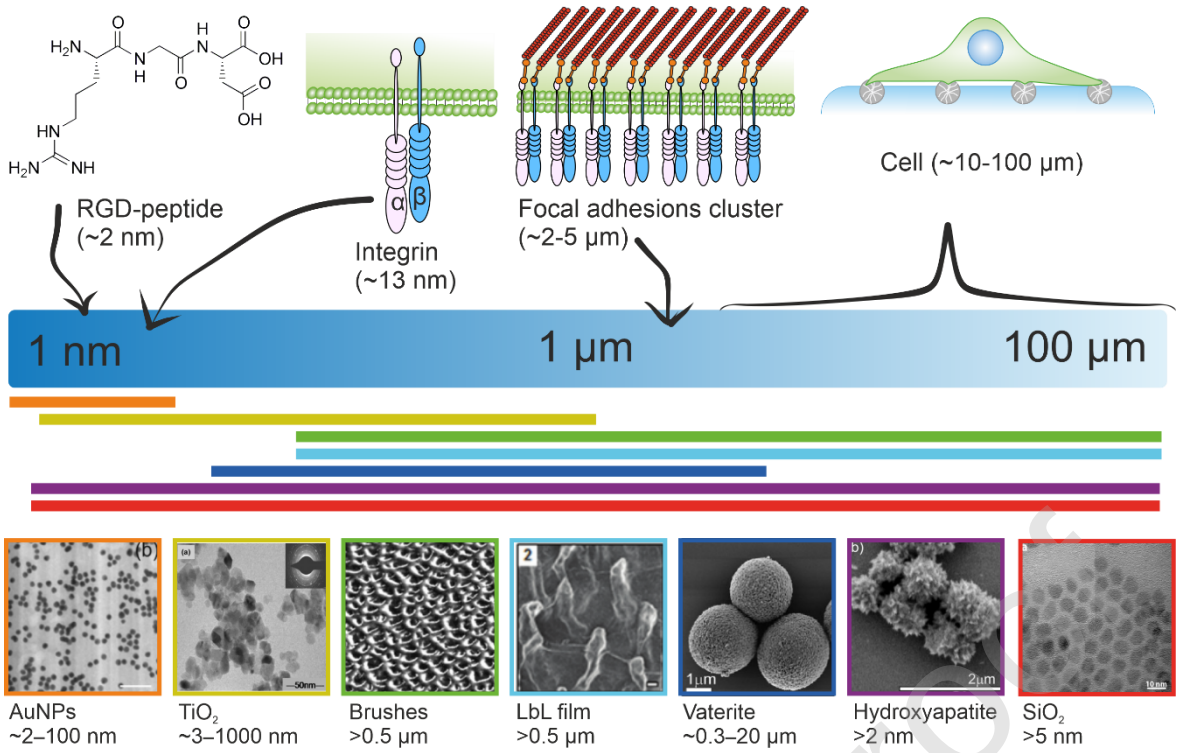


Fig. 1. Schematics presenting an overview of hybrid coatings composed of hydrogels and colloids. The pink and blue zones show synthetic and natural polymers, respectively.

Developing hybrid materials by surface modification of hydrogel using polymers, nano- and microparticles is a growing field for biological research and for medical use.[38–41] One of the main challenges in developing matrixes for cells is a lack of knowledge about the regulation of cellular behaviour by hybrid material. For example, the spatial position of nanoparticles on the surface of a hydrogel and the strength of the bond between the particles and the hydrogel matrices can affect cell adhesion.[42,43].

Figure 2 demonstrates biological processes on a scale of length from nanometers to hundreds of micrometres. First important component of adhesion process is RGD -peptide. The size of the RGD peptide molecule is several nanometers. Cell using the integrin head (Integrins are a family of transmembrane heterodimeric adhesion receptors that biochemically and mechanically interact with specific ECM components.[44]) is about 13 nanometers in size, attached to the surface. The mature integrin complex is from 1 to 5 micrometres, and the whole cell body is 10-100 micrometres, depending on the type of cell.[44–46]. There are many materials for modifying hydrogels with poor cell adhesion.[1] At the same time, the size of the colloids used for modifications can range from several nanometers to hundreds of microns and even more, thereby not only cell adhesion, morphology, survival, and even integrin clustering can be regulated.[29,47]



AuNPs ~2–100 nm | TiO_2 ~3–1000 nm | Brushes >0.5 μm | LbL film >0.5 μm | Vaterite ~0.3–20 μm | Hydroxyapatite >2 nm | SiO_2 >5 nm

Fig. 2. Length scales for cellular components, and size distribution of polymers, micro/nanoscale particles. TEM image of AuNPs reprinted with permission from ACS.[48] TEM image of TiO2 reprinted with permission from Elsevier.[49] AFM image of the brush polymer film reprinted with permission from Frontiers.[29] SEM image of LbL film reprinted with permission from RCS. [50] SEM image of vaterite particles reprinted with permission from Wiley.[51] SEM image of hydroxyapatite particles reprinted with permission from Elsevier. [52] TEM image of SiO2 reprinted with permission from Wiley.[53]

Cell-matrix interactions

To better understand the technology for preparing hybrid materials for cell adhesion, we need to discuss the interaction of cells with natural surfaces.[54] Cell adhesion is a crucial process in intracellular and extracellular interactions.[42] During the initial interaction, cell receptors bind to cell-adhesive ligands (peptide or glycan ligands). These ligands are often short polypeptide sequences in ECM proteins that bind to integrin and form adhesions for cell attachment.[55] Upon forming complex linkages with the cellular cytoskeleton, integrins mechanically pull on the ligands, and this cell-generated force from actomyosin contraction is balanced by ECM resistance and surrounding cells. Then the cell increases its area due to the movement of lamellipodia **(Fig. 3a)**. Lamellipodia have a length of two to four micrometres. At the end of the lamellipodia, there are filopodia, which are protrusions several microns long and several hundred nanometers wide.[56] Their goal is the search for cell-adhesive ligands in the environment. At this point, integrin clusters form. These clusters bind to actin microfilaments, which form stress fibres.[44] Such adhesions are about 250 nm in size and contain only paxillin and talin. With increasing mechanical stress, the size of the integrin complex increases to 500 nm. Complexes of this size incorporate α-actin and focal adhesion kinase (FAK). When the complex becomes 1-5 microns in size, it is considered to be a mature focal adhesion and contains vinculin and zyxin **(Fig. 3b-c)**. Biochemical and mechanical signals about focal adhesion are transmitted to the rest of the cell and modulate its further behaviour: motility, morphology, size, polarity, adhesion and proliferation.[57,58]

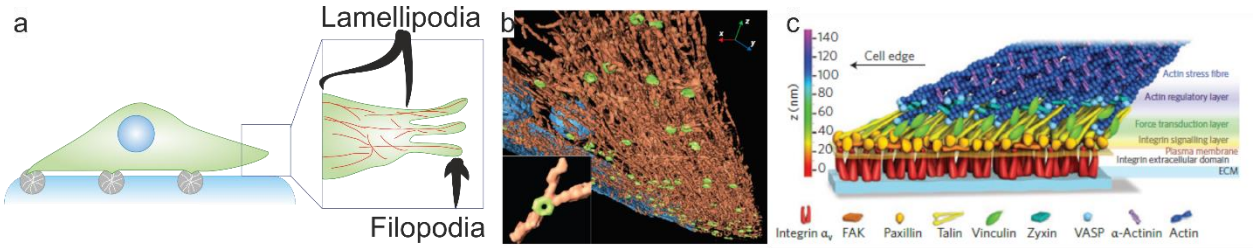


Fig. 3. (a) Cell spreading and searching for adhesive areas (dark ovals) using sheet-like lamellipodium and finger-like filopodia. (b) The nanoscale structure of focal adhesions, and nanoscale connectivity to the cytoskeleton. An adhesion-related particle (green) at sites of focal adhesions. These particles can fit between gathered integrins and link nanoscale actin to microscale stress fibres. The inset shows a close-up of a particle. (c) The nanoscale structure of a focal adhesion showing the integrin/extracellular binding domain, integrin signalling layer, force transduction layer and actin regulatory layer. Figures reproduced with permission from Nature. [38]

CD44 and receptor for HA-mediated motility (RHAMM) are the two main HA-receptors whose biological functions in human and murine inflammations and tumour cells have been investigated comprehensively. HA was initially considered to be only an inert component of connective tissues but is now known as a "dynamic" molecule with the constant turnover in many tissues through rapid metabolism that involves HA molecules of various sizes: high molecular weight HA (HMW HA), low molecular weight HA, and oligosaccharides. Such receptor systems help cells anchor on the surface of biomaterials but do not give such a strong connection as integrin molecules.[59]

Also, cells, such as white blood cells, might use nonspecific and weak electrostatic interactions of glycosylated cell surface molecules with collagen fibres and other ECM components. These interactions might generate friction and thereby enable the transmission of forces generated by the cell to the ECM.[60]

Cell adhesion on hydrogels functionalised with polymers

A prerequisite for studying molecularly defined cell adhesion is the availability of a nonadhesive, passivated background surface that enables the attribution of specific cellular responses entirely to the interaction of the particular cell surface receptors with specific adhesion mediating ligands. Among these, polyethene glycol (PEG) and alginate-based substrates are widely used as surfaces without any ligands for cells adhesion.[61]

The creation of branched PEG-based copolymers is an attractive method for functionalising the surface of a PEG hydrogel. The most common branched PEG materials are based on poly (oligoethylene glycol methacrylate) (POEGMA), which consists of a methacrylate backbone and one PEG side chain of tunable length per monomer repeat unit. This structure provides easy polymerisation through a free radical. Lutz and coworkers demonstrated that thermoresponsive oligo(ethylene glycol)-based gold surfaces allow efficient control over cell-adhesion within a convenient and applicable temperature range (25–37 °C).[62] Thus, these novel smart substrates advantageously combine some features of PNIPAM surfaces (i.e. switchability) and PEG surfaces (i.e. bio-repellency at room temperature) **(Fig. 4)**. These findings open new avenues for the design of advanced functional surfaces for cell culture engineering, bioseparation, and diagnostics applications (Fig. 3).

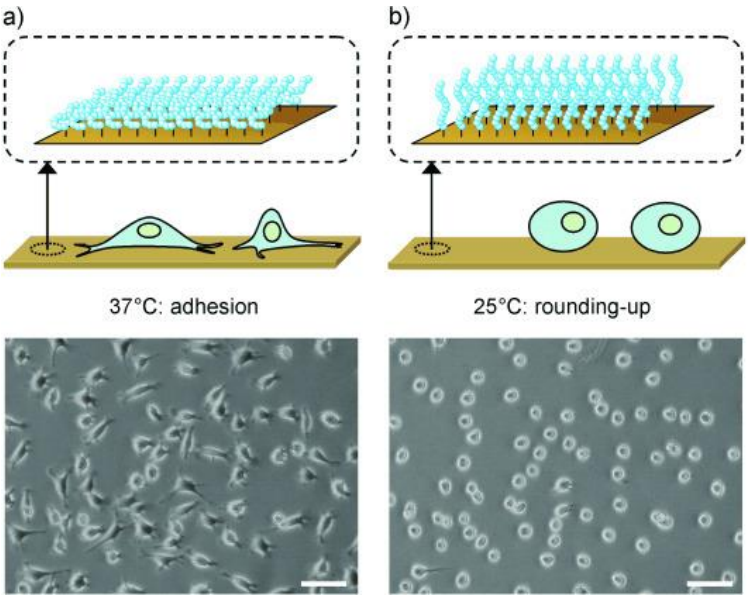


Fig. 4. Phase-contrast microscopy images of fibroblasts on poly(OEGMA-co-MEO2MA)-modified gold substrates after 44 h of (a) incubation at 37 °C and (b) 30 min after cooling the sample to 25 °C. Reprinted with permission from Wiley. [62]

Layer-by-layer (LbL) self-assembly technique is a simple and commonly used method to realise surface modification, which has attracted much attention because of its simplicity in procedure, wide choice of materials and fine-tuning of the microstructure.[63] LbL assembled multilayer is fabricated by sequential adsorption of materials with complementary functional groups employing electrostatic interactions, hydrogen bonding, or covalent interactions.[64] The LBL method is extremely simple for applying it to hydrogels.[40] This technique has been broadly used to modify the surface of biomaterials through deposition of biocompatible multilayers which could also release functional molecules upon stimuli response.[65,66] This method does not destroy the architecture of hydrogels and significantly increases its functionalisation.[67] The preliminary work on LbL modification of hydrogels was presented by Sakaguchi *et al.*, in which PVA hydrogel was used as the template and coated with dextran sulfate and chitosan in an LbL fashion.[68]

Modification of LBL hydrogels by polymers affects hydrogel parameters such as mechanical properties[69–72] and surface charge.[73,74] These parameters primarily affect cell adhesion. Between the layers, binding sites for cells can be fixed.[75,76]

P. Gentile at al. developed functionalised hydrogels at the nanoscale by LbL assembly to promote cartilage healing **(Fig. 5)**. Hydrogels, based on sodium alginate and gelatin, were prepared by an external gelation method consisting of $CaCl_2$ diffusion and genipin addition for cross-linking (G cross-linking method). Genpin is the aglycone of geniposide, which increased water stability of scaffolds as well as the mechanical properties under compression.[77] Due to easy degradability of gelatin *in vivo,* they are improved stability and mechanical strength by parallel cross-linking of alginate/gelatin mixture. Successively, hydrogels were coated with G to obtain a positive charge on the surface, then functionalised by LbL assembly to create 16 nanolayers, based on poly (styrene sulfonate)/poly (allyl amine) (PSS/PAH), including a specific peptide sequence and transforming growth factors β1. The presence of a biologically active LbL film over the hydrogel surface improved chondroblast proliferation and glycosaminoglycan secretion at the nanoscale.[10]

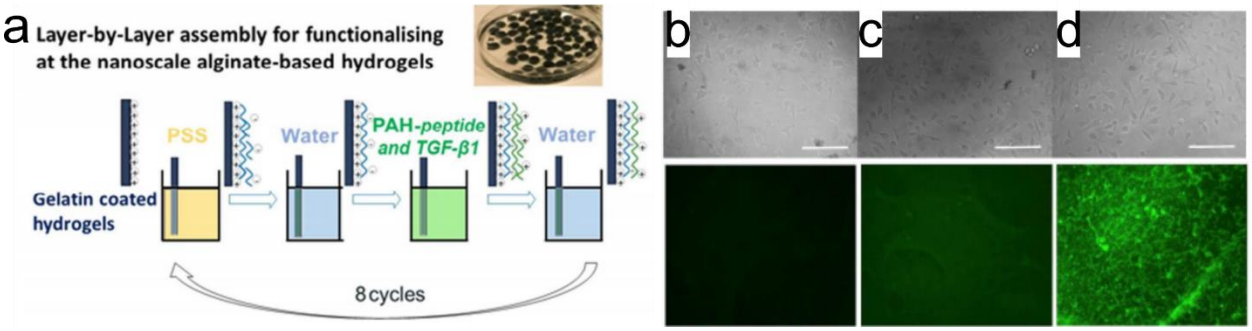


Fig. 5. (a) Schematic view of the alginate hydrogels coating (b-c) Optical and fluorescence microscopy images of (b) AG5%, (c) LbL functionalised hydrogels without biomolecules addition, and (d) LbL functionalised hydrogels with the addition of FITC- CTATVHL peptide and TGF-β1. Bar= 100 µm. Reprinted with permission from RCS. [10]

One more class of hydrogel films functionalisation is presented by surface-grafted polymer networks (polymer gels or covalently cross-linked hydrogel brushes), which possess not only some of the characteristics of polymer brushes but also tunable swelling and mechanical properties. Polymer gel brushes were previously prepared either in situ, by surface-initiated polymerisation in the presence of cross-linker, or ex-situ, by postmodification of pre synthesised polymer brushes.[78–81]

Also, as functionalisation of the surface of biomaterials, including hydrogels, polymer microcapsules can be used.[82] They can have a dual function: improve cell adhesion and be carriers of bioactive molecules, the release of which can be stimulated by ultrasound or laser.[83–85]

Cell adhesion on hydrogels functionalised with nanoparticles

Carbon-based nanoparticles (carbon nanotubes),[86–88] polymer nanoparticles (hyper-branched polymers),[19,89] inorganic particles (silicates),[90,91] ceramic nanoparticles (hydroxyappatite and calcium phosphate),[92,93] metal nanoparticles (gold, silver and iron)[94,95] are widely used to modify hydrogels: 1) response to various stimuli such as light,[96] electricity,[97] magnetic field,[98] ultrasound;[99] 2) improvement of mechanical properties;[21] 3) creating a three-dimensional relief on the surface; 4) improved cell adhesion.[100]

Spark et al. studied the behavior of cells on a nanostructured material with emphasis on the fundamental adhesive properties of cells. Authors used a nanocolloidal assembly to study the possible allowable distance between nanoparticles for cell adhesion. For this reason, gold nanoparticle incorporated to PEG-hydrogel with the controllable distance between them (Fig.6). The size of the gold nanoparticles was 8 nm, which allowed only one integrin molecule to bind to the particle surface. They showed that when adhesive dots are separated by ≥73 nm, cell adhesion and spreading, as well as the formation of focal adhesions, are aberrant, whereas separation of ≤58 nm between the dots allows effective adhesion. This feature is not attributable to the insufficient number of ligand molecules but to the restriction of integrin clustering. Spark et al. found the maximum spacing between nanoparticles occupied integrins necessary for adhesion and focal adhesion formation to be between 58 and 73 nm, not just for MC3T3-osteoblasts, but also for other cells cultures.[101]

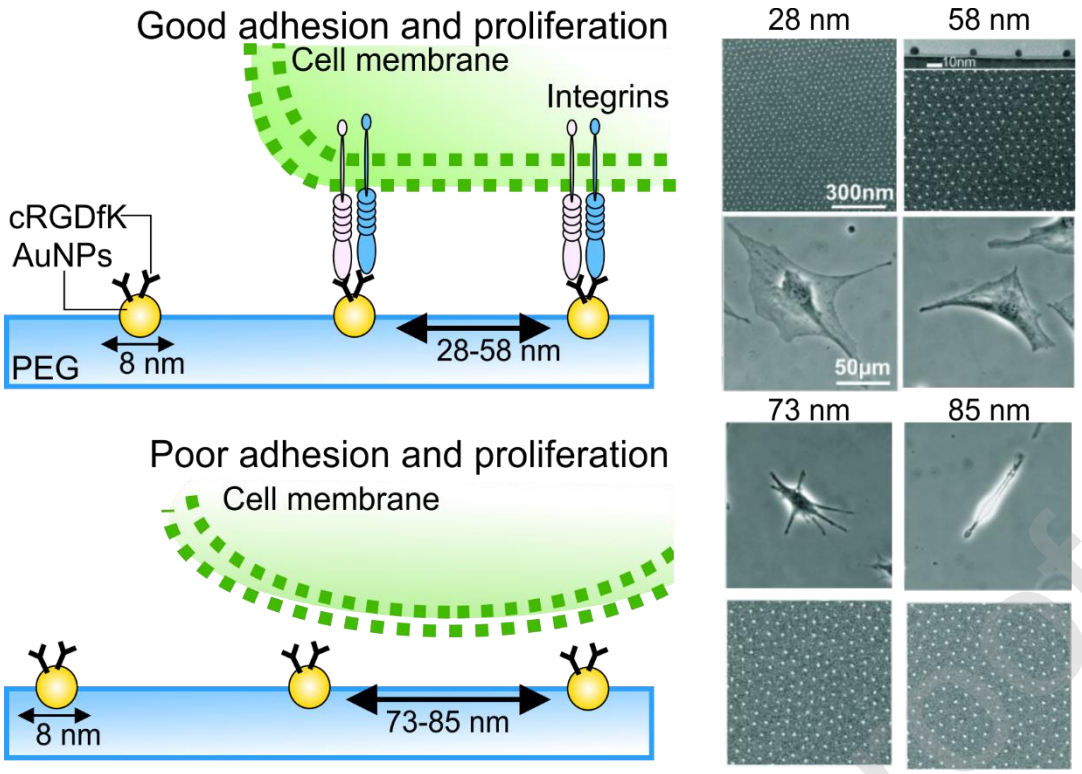


Fig. 6. A schematic of a biofunctionalised gold particle substrate in contact with a cell membrane (left panel) and a scanning electron micrograph of PEG+AuNPs surface and light microscopy of a cell that is adhering to a gold particle (right panel). Reprinted with permission from Wiley. [101]

Especially gold nanoparticles (AuNPs), which have unique size- and shape-dependent optical properties via surface plasmons, little toxicity, easy synthesis procedures, are desired materials not only for industry, and catalysis but also for biology and medicine.[102,103] For providing cell adhesion on the bio-inert PEG surfaces, several micro-fabrication techniques have been developed to modify the surface properties and to promote cellular adhesion; for example, applying patterns of surface nano- or microtopographies, patterns of elasticities, or chemical modifications. A good example of modifying the surface of a hydrogel with gold nanoparticles is an article by Yesildag *et al.* [94] They used a micro-contact printing system to create uniform coatings of gold nanoparticles on a PEG hydrogel **(Fig. 7)**. They examined adhesion on the L929 fibroblast cell line. Figure 3 clearly shows that fibroblasts adhere exclusively to gold nanoparticles. This allows selective control of adhesion on the surface of the biomaterial. Due to such selective adhesion, it is possible to create tissue-engineering structures of the required configuration.

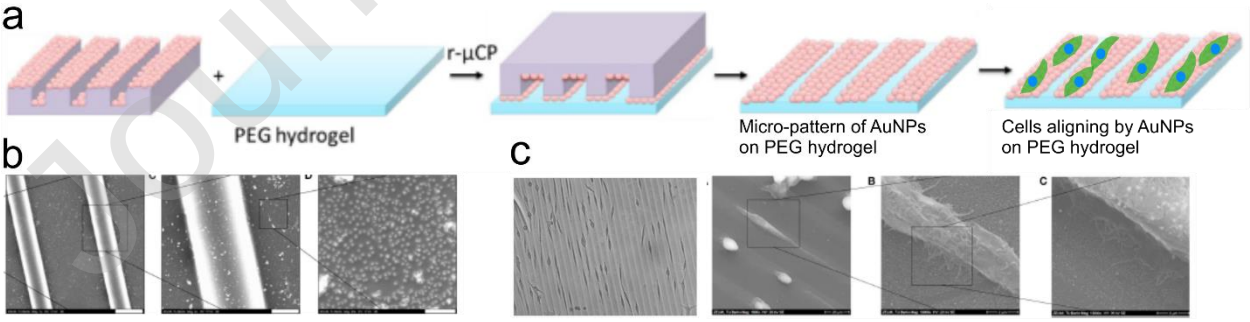


Fig. 7. (a) Schematic view of reactive micro-contact printing (r-µCP) process of Au NPs on PEG hydrogels. (b) SEM images of amino-silanised PDMS-stamp (used master sizes was [20–10–5µm]) inked with Au NPs. (c) SEM images of an aligned and stretched cell on the AuNPs micro-pattern. Reprinted with permission from Frontiers, 2019 [94]

Cell adhesion on hydrogels functionalised with microparticles

One of the most striking examples of microparticles for cell adhesion is calcium carbonate particles. Calcium carbonate has three anhydrous states: vaterite, calcite and aragonite, and over time can pass from one form to another.[51,104,105] Especially attractive is spherical porous vaterite particles, which can be pre synthesised with the following adsorption on the hydrogel [] as well as synthesised directly on the surface of various biomaterials.[25] The porosity of the vaterite particles makes them attractive to load various substances in them, for example, bioactive molecules and drugs.[106] In the process of recrystallisation, from unstable porous spherical vaterite, the phase of smooth calcite stimulate the payload release. The additional coating increases the hydrophilicity, which significantly improves cell adhesion.[107] **(Fig. 8)**. The primary adhesion of cells occurs through a contact of hyaluronan with $CaCO_3$ surface.[108,109] Hyaluronan is a large, linear glycosaminoglycan composed of a repeating disaccharide of glucuronic acid and N-acetylglucosamine. Its large dimensions render it an excellent candidate for long-range interactions of cells with external surfaces. It is presumed that one single molecule of hyaluronan may extend to several microns from the surface to which it is tethered or adsorbed. Hyaluronan synthesised on the membrane of a cell by hyaluronan synthetase and connected to CD44.[110] Due to the big size of hyaluronan, it works as an anchor and can connect with $CaCO_3$ surface a couple of micrometres away **(Fig. 8b)** after that cell emits ligands (as fibronectin) and connects with $CaCO_3$ by integrin, leading to a fully spreading and structural organisation **(Fig. 8c)**.

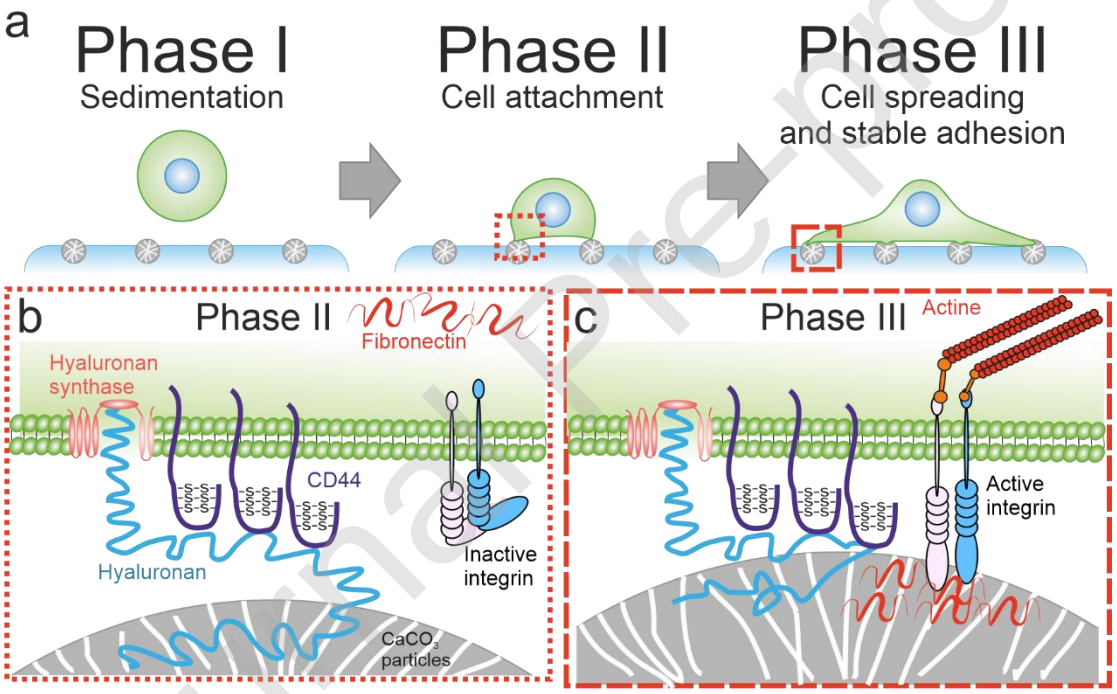


Fig. 8. (a) Phases of *in vitro* cell adhesion. (b) Detailed schematic of the adhesion process on $CaCO_3$ particles in phase II (c) Detailed schematic of the adhesion process on $CaCO_3$ particles in phase III.

Abalymov et al. in their work studied the possibility of modifying alginate hydrogels with vaterite $CaCO_3$ particles as a result of biomineralisation (Fig. 6). Alginate gels do not allow cell attachment in the absence of cell-adhesive ligands and are relatively biocompatible, and they, therefore, offer a tunable system in which cells attach only where cell-adhesive ligands are presented. Alginate hydrogel underwent thermal annealing (T-A), which significantly improved its mechanical properties in seven times **(Fig. 9)**. After that, the hydrogels were mineralised. However, the mechanical properties of hybrid hydrogels were obtained by AFM and showed no statistical difference between Young's modulus of the vaterite particles of regular alginate hydrogel and T-A alginate hydrogel. It turned out that cells can adhere well only for mineralised T-A alginate. Such difference between cells adhesion on the particles incorporated to soft and hard hydrogels shows mechanosensitive properties of integrin connections with colloidal particles. In the case

of regular alginate hydrogels, cells anchored with particles by hyaluronan, but the formation of stable connection by integrin is impossible, because of the movement of particles in the soft matrix during the formation of stress fibres. [43]

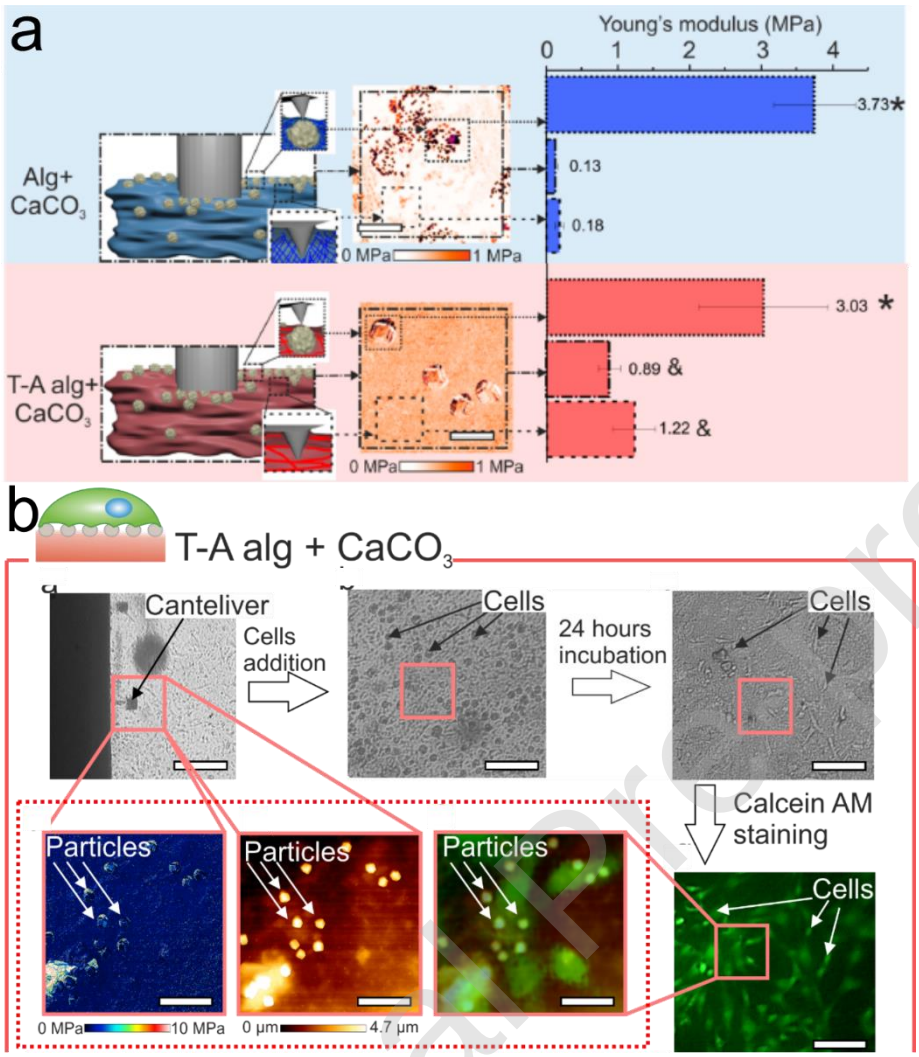


Fig. 9. (a) Young modulus values for alginate hydrogel and T-A alginate hydrogel obtained using Universal Testing Machine (1 mm tip) and AFM (scale bar is 4 µm). (b) Combined monitoring of cell adhesion on TA alginate hydrogels using AFM and confocal microscopy. Reprinted with permission from ACS, 2020. [43]

However, the connection between hydrogel and particles is not only single possible factor that influences cell focal adhesion, proliferation, and ossification (the formation of an ECM consisting of proteins and hydroxyapatite, which is similar to bone). In the next work, authors varied by a ratio of $CaCl_2$, Mg_2CO_3, and Na_2CO_3 in the reaction mixture for mineralisation of T-A Gellan Gum hydrogel. Particles obtained on the hydrogel surface as a result of mineralisation have different size, morphology, mass, and mechanical properties **(Fig. 10)**. These factors have different effect on cell density, area, viability, alkaline phosphatase, and hydroxyapatite synthesis **(Fig. 10a)**. The authors showed that all of the above factors affect the ability of cells to adhere to the surface. Primary adhesion is most affected by Young's modulus of the particles (YmP) and the percentage of calcium in the synthesis **(Fig. 10b)**, their area (spreading and stable adhesion) is most affected by the amount of mineral, the YmP of the particles, and the percentage of calcium **(Fig. 10c)**. Obviously, the YmP, and as a consequence, their combination with the hydrogel matrix, has a positive effect on cell adhesion. Particle mass is also an important parameter, since the more

particles, the more binding sites for cell ligands. Cell viability is affected by a combination of all these factors **(Fig. 10d)**.

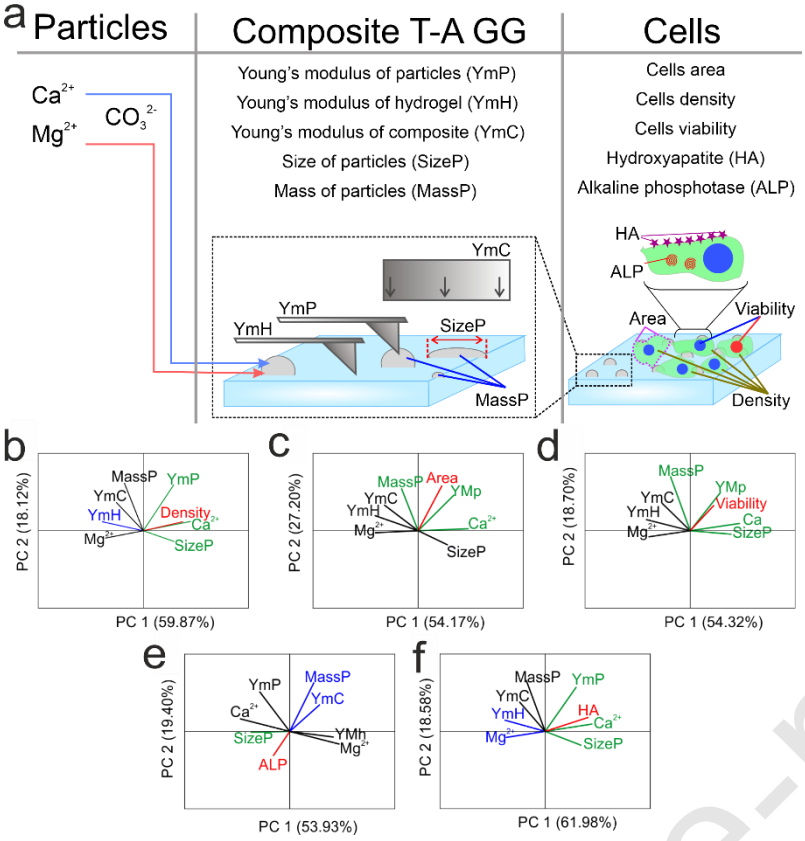


Fig. 10. (a) Schematic representation of major factors, which influenced cells ossification was analysed. The first column represents the initial technical parameters of gel mineralisation like the ratio of the calcium and magnesium ions. The second column represents the characterisation of the designed hybrid materials. The third column represents the factors related to the growth and activity of the cells. (b) Loading plot of the first two PC loading vectors: Mg^{2+}, Ca^{2+}, YmC, YmP, YmH, MassP, Area, Viability, SizeP, ALP, Density, HA. In red colour highlighted the factor of interest. The significance of the influence on the factor of interest is highlighted in various colours: significant positive influence (green); negative influence(blue) doesn't have a significant influence (black).

Muderrisoglu *et al.* also used a combination of alginate and addition of $CaCO_3$, and modified this system with alkaline phosphatase (ALP) molecules.[9] This one was also adsorbed onto the surface of the hydrogel. Due to the additional surface area, carbonate particles significantly increased the amount of enzyme, thereby improving the production of the extracellular matrix consisting of hydroxyapatite, which is one of the main components of the bone i.e. cells ossification. A plus of this system is the triplicity of vaterite particles, which are both binding sites for cells, containers for bioactive molecules, and a source of calcium ions. It should be noted that these particles can be not only synthesised on the surface of the material, but also obtained from a separate synthesis, modified separately, and after that built into the desired hydrogel. Abalymov et al. used vaterite particles into which ALP molecules were loaded. Such carriers are both a container and a building material for the extracellular matrix, thereby greatly accelerating the production of hydroxyapatite on the surface of osteoblasts.[111]

Conclusion and outlook

In conclusion, we have overviewed state-of-art and highlighted recent developments in the area of biomaterial and hydrogel modification by colloidal nanoparticles. Such a modification represents an effective way of designing new surfaces with improved cell adhesion capabilities. Modification of soft, organic coatings by inorganic micro- and nano- particles and colloids leads to the formation of the so-

called hybrid materials, where the two phases: organic and inorganic complement each other. Such hybrid materials have been shown to enhance, and most importantly, control mechanical properties and the Young's modulus of the coating, thus allowing to adjust them to match for any cell type. In addition to the adjustment of mechanical properties, the incorporated particles allow for a controlled release of encapsulated biomolecules, which further extend the range of functionalities of such hybrid coatings. Cell adhesion – one of the key applications of these coatings – has been enhanced and controlled due to these coatings.

In future, it is expected that other types of particles will be used for the surface functionalisation. Remote release and remote-control functionalities are projected to be developed and applied for a controlled release of relevant molecules. Eventually, various cell types will be seeded, allowing a broad application of such coatings in tissue engineering and biomedicine.

Credit author statement

Anatolii Abalymov: Conceptualization, Writing- Original draft preparation. **Bogdan Parakhonskiy**: Reviewing and Editing. **Andre Skirtach**: Reviewing and Editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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