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Biomaterial-stem cell interactions and its impact on stem cell response

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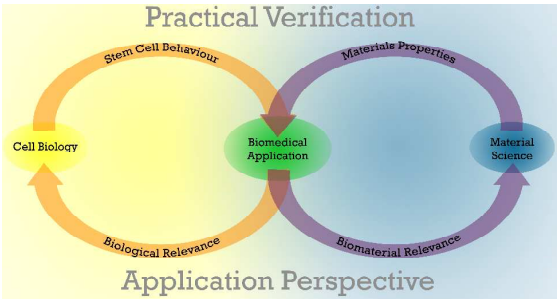

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In this review, current research in the field of biomaterial properties for directing stem cells are discussed and placed in a critical perspective. Regenerative medicine, in which stem cells play a crucial role, has become an interdisciplinary field between cell biology and material science. New insights are generated, different approaches to determine material features and stem cell properties are implemented, but also many misconceptions exist. According to the current state-of-the-art and combined with basic principles from two different disciplines the topic is critically addressed. We take in to account what seem to be the most important material properties and their influence towards stem cells but also the various stem cells available with respect to their origin, tissue source and culturing conditions.

Keywords: biomaterials; biointerfaces; hydrogels; stem cells; self-renewal; multilineage differentiation; tissue engineering; regenerative medicine.

Introduction

Defined scaffolds are of key importance for studying different aspects of stem cell activity in order to maximize the full potential of stem cells in regenerative medicine and tissue engineering.[1–3] Although, much complexity of stem cell function depends on the chemical and mechanical properties of a given substrate, the origin of the applied stem cell with respect to both developmental stage and tissue type are just as important. So far, the focus has been mainly on interactions between biomaterials and mesenchymal stem cells (MSCs)[4] (reviewed by MacQueen *et al.*[5]). Responses of other stem cells such as embryonic stem cells (ESCs), hematopoietic stem cells (HSCs) and induced pluripotent stem cells (iPSCs) with various biomaterials have been less thoroughly and critically addressed and compared. In this review, we particularly discuss and compare responses of embryonic and adult stem cells to physical and chemical features of reported biomaterials from both chemical and biological points of view. This provides a deeper insight into the effects of mechanical and chemical properties of various synthetic substrates on both morphological and functional properties of stem cells of different origin.[6–8] Interactions of these stem cells with the same synthetic substrate may lead to stem cell origin-specific changes in their properties but also differences between stem cell sources and even within one cell type are observed.[6,7] Furthermore, culturing stem cells on biomaterials of various dimensionality can result in differences in adhesion, self-renewal and differentiation.[7,9,10] Therefore, comparing responses of different stem cells to the same biomaterial under the same conditions but also the same stem cell to different biomaterials with similar as well as deviating properties need to be taken into account. This is important to elucidate the parameters, which appear to be essential for not only a proper biomaterial selection, but also determining the most appropriate stem cell type for optimal usage for both regenerative medicine and tissue engineering purposes. It has been reported that developmentally related stem cells can respond in different or even opposite manner to the same substrate. Therefore, in order to choose a proper experimental design for a particular stem cell type and a given biomaterial, the most time-efficient and practical approach would be first to examine basic biomaterial compatibility parameters such as stem cell viability and apoptosis. These parameters have been analysed very scarcely and have randomly been reported for biomaterials used for stem cells cultures. Thus, a systematic analysis of biomaterial stem

cell compatibility is still lacking. Markedly, a few studies have been performed using a high throughput screening with combinatorial approaches such as biomaterial arrays,[11–13] where separate combinations of chemical compounds, (surface) topography or growth area were tested mainly for one stem cell type. Even though, high throughput approaches reduce labor-intensiveness, still it can become quite expensive. Especially, combinatorial approaches including generating different nano-sized topography combinations by lithography remain still very expensive but still remains the most powerful method for creating defined topographies so far. Therefore, there is a growing interest in reducing labour-intensive and costly investigations of (stem) cells towards a particular biomaterial by exploiting mechano-, chemo-, and topo-sensitivity by designing substrates with combined parameters that gradually change within the same substrate.[14–17] For example a stiffness combined with a polarity gradient can be a highly suitable approach to design biomaterials as it can be used to determine optimal combinations of both chemical and mechanical parameters for stem cells. Further, as recently reported instead of using animal-derived adhesion proteins or ECM components for coating biomaterials,[12] the human serum or recombinant human adhesive proteins should be used to properly study interactions of a particular biomaterial with human stem cells. Also recently developed ECM mimics appear to be promising components for coating biomaterials (reviewed by Wade *et al.*[18]), and hence could be used to target the adhesion and growth of stem cells. Coating approaches are in line with many ongoing investigations and seem to be a convenient approach to influence stem cell adhesion. However, it is hardly ever mentioned or investigated what the true origin of the measured effect is since surfaces are covered by adsorbed proteins, which are affected by different surface properties and serum composition as well. Therefore, to maximize the functional potential of a given stem cell type, the single biomaterial with a gradient of mechanical and chemical parameters, and coated by adhesion protein(s) of the same species and / or developmental origin as the applied stem cells, is the optimal strategy. These approaches can be considered as making a complex investigation even more complex but in order to achieve the next major breakthrough in stem cell-based therapies including tissue engineering and regenerative medicine, approaches need to include: 1) more complexity by combining parameters; 2) more standardized approaches without any ambiguity both from the material and biological point of view; 3) the definition of the "true" origin of observed effects (chemical- or biological factors and adsorbed proteins); and 4) a broadening of the stem cell spectrum by including stem cells of different origin and genetic disposition in the same investigation in order to determine generality or deviations in stem cell behaviour.

It has already been stated by others that defining stem cell behaviour including morphological characteristics as well function is highly challenging due to the non-standardized approaches for such studies.[19] However, by introducing biomaterials in the equation the standardization should be extended also towards the material aspect in terms of: 1) determining contributions of surface properties with respect to bulk properties (mechanical contributions); 2) chemical contributions by defining more appropriately the origin of the observed effects (surface chemistry); 3) physicochemical aspects concerning the overall protein behaviour at differently designed interfaces; and of course 4) the contribution of more biological settings such as cell culture media composition, substrate pre-treatment, sterilization protocols etc. Therefore, when approaching such an interdisciplinary and highly multi-parameter system, a standardized approach from both cell biology as well as the material/chemical science is needed to efficiently assimilate and appropriately connect knowledge obtained from different studies (**Figure 1**). In addition to the cell biology aspects also biological mechanisms such as signalling pathways, transcription factor regulatory networks, molecular signatures (*i.e.* gene expression profiling) leading to observed stem cell behaviour should be elucidated and covered as the molecular response of stem cells to a given biomaterial. However, so far the molecular response of stem cells has been only thoroughly reported for TCP-based culture system and not for other biomaterials. Therefore, instead of 'high-throughput' testing of different materials and scarcely analysing biological functions of applied stem cell, the multidisciplinary experimental design for biomaterial-stem cell research should in the future also focus on both detailed cellular and molecular characteristics of stem cell activity. Further, the discussion in this review mostly concerns polymeric materials, the general idea on how materials-stem cell research focus should change applies to all biomaterials including ceramics and metals.

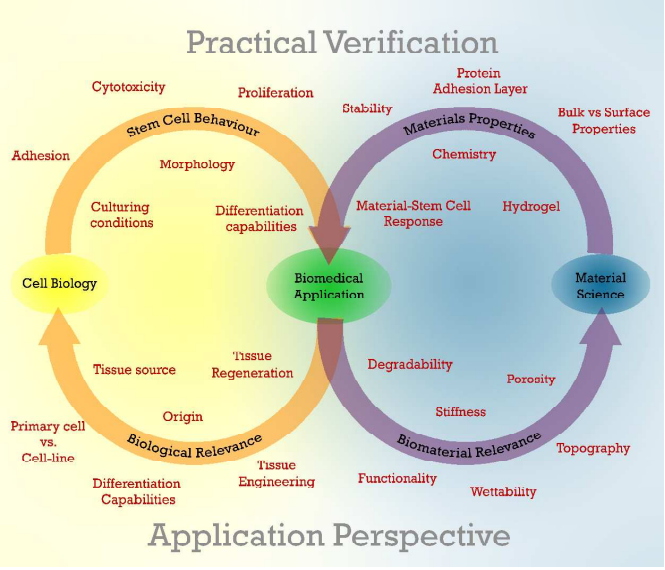


Figure 1: To achieve a general consensus about stem cell response towards biomaterials, new methodologies and a broader scope of investigations need to be performed. From the main biomedical application which is addressed, an initial discrimination on both stem cell behaviour as well as functional material can be made based on the knowledge generated so far. However, the practical verification whether the biomedical application can be targeted, a broad and rigorous investigation is vital encompassing both the cellular as well as molecular changes needs to be included but also in between analysis and characterization of the materials properties which will surely be affected when performing experimental procedures.

Environment-sensitivity of stem cells

During different stages of mammalian development, various types of stem cells such as embryonic stem cells (ESCs), hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) have been isolated. All of these stem cells are defined by their characteristic capacity to both self-renew and differentiate.[20–22] ESCs, derived from the inner cell mass of the mammalian blastocyst, are the most potent (so called pluripotent), are able to self-renew indefinitely and give rise to all tissues (reviewed by Rippon *et al.*, Chambers *et al.* and Keller [21,23,24]). Similar properties have recently been described for the induced pluripotent stem cells (iPSCs) (reviewed by Inoue *et al.* and Takahashi *et al.*[25,26]), which originally were produced from adult skin fibroblasts by genetic reprogramming.[27] In contrast to the ESCs and iPSCs with their potency, both HSCs and MSCs are described as multipotent stem cells, which can self-renew and differentiate for a limited time into all different cell types contributing mainly to one tissue (reviewed by Jaenisch *et al.* and Krampera *et al.*[28,29]). In addition, MSCs and HSCs have been found in different tissues and / or organs within the developing embryo and in the adult [30–32](reviewed by Wilson *et al.* and Dzierzak *et al.*[22,33]). In contrast to the *in vitro* suspension liquid cultures typical for non-adherent HSCs, MSCs are able to grow and differentiate only when adhered to a solid. Nevertheless, all these stem cells, similarly to previously reported differentiated cells, are able to sense physical, chemical and dimensional cues of a given environment [4,6,34] (reviewed by Higuchi *et al.*[35]). It has even been shown that MSCs can sense the stiffness of a hidden underlying stiffer layer of less than 5 µm beneath a polyacrylamide (pAAm) gel without having a direct contact to it.[36] This ability stresses the importance of biomaterials design with highly defined and preferably tuneable properties in order to direct the stem cell fate.

Stem cells are binding to the extracellular matrix (ECM) via adhesion molecules (i.e. integrin- based adhesion complexes). These transmembrane adhesion complexes are known to be tightly associated with the actin-myosin cytoskeleton, which in return receives a mechanical feedback from the ECM leading to a change in the cytoskeleton organization and thus altered stem cell shape (reviewed by DeSimone *et al*. and Geiger *et al.*[37,38]) This feedback results ultimately in changes in stem cell fate decisions comprising self-renewal, differentiation, migration and apoptosis. Therefore, the ECM is one of the most essential components of the microenvironment, regulating the stem cell-niche/substrate interactions. Depending on the stem cell niche and/or substrate properties, the ECM may undergo remodelling resulting in modified physical parameters. This process of ECM remodelling, often described as ECM contractions, might lead to locally enhanced contact between stem cells and /or between stem cells and other cells, followed by increased intercellular contact-dependent signalling or paracrine signalling. Such ECM contractions have been observed and reported during mammalian development, for example during organogenesis.[37] Notably, also during *in vitro* differentiation of human MSCs time-dependent changes in the ECM elasticity play a role. Earlier ECM stiffening promotes osteogenic differentiation of MSCs contrary to late ECM stiffening favouring adipogenesis.[39]

Following development of the mouse, microenvironment(s) nurturing stem cells undergo many anatomical and stress-related changes. While little is known about the physical properties of embryonic microenvironments harbouring stem cells, the murine adult bone marrow (BM) remains so far the best characterized microenvironment both biologically and physically. Within the BM niche, the elasticity (also referred to as stiffness and most often expressed as the Young's modulus) varies significantly, ranging from the most peripherally located rigid mineralized bone (>10^6kPa) to a relatively soft (<0.3 kPa) central marrow.[40] Therefore, attempts to create artificial niches mimicking the native BM-niche for HSCs and MSCs with the use of biomaterials of different stiffness have been investigated.

In addition to the mechanical properties of biomaterials, other properties such as wettability, (surface) topography, porosity and surface chemistry also need to be investigated with respect to the interactions between stem cells and the substrate. These surface parameters have been identified to be highly influential for stem cell adhesion and fate, but many questions still are unanswered concerning the details or origin of the measured effects and how the various parameters influence each other. Especially, the latter will have a significant impact since a surface always presents multiple parameters simultaneously. One of the simplest approaches so far has been identifying how wettability influences stem cell adhesion, proliferation and differentiation. First of all, the stem cells will not directly respond to the surface but instead indirectly via a protein layer adsorbed onto the surface.[41] This protein layer dictates the surface interactions by composition and protein orientation / conformation which depends on the wettability.[42,43] However, the underlying chemistry consisting of either non-charged polar surface groups or positive and negative charges will affect this layer as well. Therefore the stem cell origin combined with the origin of the surface parameters will make the endeavour more complex than it already is. Still, it is absolutely necessary to take all these parameters into account in order to reach a higher level of understanding towards biointerfaces. This is from the materials perspective combined with cellular behaviour regarding proliferation, toxicity, differentiation and adhesion however in order to obtain the full scope, it should also be complemented by a fundamental mechanistic approach.

Since most studies have analysed stem cell fate regulation by a given substrate with the use of MSCs, responses of other stem cell types such as ESCs, HSCs and iPSCs to chemical, physical and dimensional material parameters should be examined as well in a more systematic fashion, especially because these stem cell types hold great promise for different therapies.[44–46] Furthermore, a comparative analysis of a broad range of substrate properties will help to determine what kind of substrate(s) characteristics are ideal for maximizing the proliferative and differentiation potential of stem cells. It is also important to consider that the ideal biomaterial composition

depends on the developmental and/or tissue origin of the stem cells. One can even go as far as stating that the same stem cell type *e.g.* MSC, of different sources should be investigated and compared since genetic predisposition, age, health and other factors give rise to deviating behaviour.[47] In humans, the response can be expected to be dependent on the individual when looking at studies involving various donors.[48]

Biomaterials directing stem cell response

Along with factors to induce stem cell fate, the stem cell-biomaterial interaction is an important parameter influencing stem cell properties in culture as well as *in vivo*. These interactions and therefore the cellular activities are dependent on a variety of materials properties. Wettability, stiffness, chemical composition and topography of a biomaterial are known to affect the behaviour of cells.[8,49–51] Substrate topography has shown to be important for cell behaviour studies, but due to their complexity they are not discussed in this review in depth. Their influence has been reviewed recently in an excellent review article.[35]

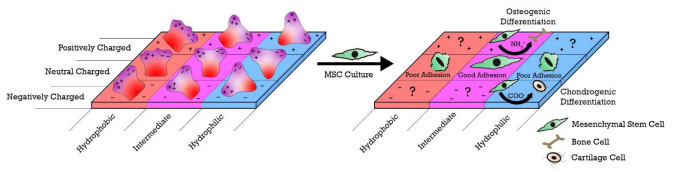


Figure 2: Illustration of adsorbed protein on surfaces with different wettabilities and different origins of wettability. On a hydrophobic surface, the protein has a higher contact area due to the tendency of denaturation at polar-apolar interfaces. In case of a hydrophilic surface, the protein will not have a contact area that high unless there is a significant amount of charge on the protein surface and thereby applying strong coulombic forces. For positively charged surfaces, negatively charged regions of the protein are also attracted to the surfaces and vice versa. These interactions have significant influences on the behaviour of MSCs as shown schematically on the right. Depending on the type of surface, MSCs will alter their differentiation pattern. Also still many combinations have not yet been tested and no uniform theory has been formulated yet.

The influence of the biomaterials properties towards cells is not always a direct influence but can also be a rather indirect one. This was shown already in 1993 by Tamada *et al.*, when was pointed out that the adhesion behaviour of cells is dependent on the pre-adsorbed protein layer on a surface.[41] Since the mobility is much higher for proteins than for cells, proteins adsorb to a biomaterial much more quickly than cells can adhere. Therefore, in a solution containing protein such as the culture medium, the (stem) cells will adhere to an already adsorbed protein layer on the biomaterials surface. Knowing this, the materials properties wettability and surface chemistry seem to influence the (stem) cell adhesion only indirectly when cultured in medium. The influence of the chemical functionality towards cell behaviour was initially observed by Lee *et al.*in 1994 but without further focus on the adsorbed protein layers.[52]They showed that chemically different surfaces, with different charges but similar wettability led to different cell adhesion. For the modulus the protein layer should not influence the biomaterials properties significantly, since a monolayer of proteins is very thin, in the order of a few nanometres. Nevertheless this indirect influence can be determined and optimised in order to control cellular behaviour. Here, we address these properties and describe still existing uncertainties concerning which properties actually direct stem cell behaviour.

Stem cells and wettability

The wettability of a materials surface is a macroscopic effect that is influenced mainly by the surface chemical functionality and topography. Proteins will adsorb in different ways onto surfaces with different wettability originating from surface chemistry.[53,54] On hydrophobic surfaces, the more hydrophobic parts of the protein will stick to the surface, in order to minimise the interaction of these parts with the aqueous phase, and therefore the protein tends to deform/denature due to rearrangements of the protein structure (**Figure 2**).[53,54]
Wettability can have significant effects on stem cell behaviour, as shown by Shin *et al.* who investigated the adhesion and proliferation of human bone marrow stem cells (hBM-MSCs) cultured on PE (polyethylene) with different surface modifications.[55] They found the highest number of adhered cells on a surface with intermediate wettability (57°-65°). The adhesion for the lowest (48°) and the highest contact angle (97°) showed decreased cell numbers. Also for the proliferation rate the maximum was found in the intermediate region. The difference in wettability in this case was created by a corona treatment of PE, which results in multiple kinds of oxygen containing functional groups on the surface. Furthermore, in a recent study by Mao *et al.* it was observed that human and mouse MSCs react to surface wettability.[56] They used a hydroxyl and a methyl terminated aliphatic thiol in self-assembling monolayers (SAM) to modify gold surfaces. The wettability was adjusted by changing the mixing ratio of both thiols, yielding contact angles from 23° till 107°. It was shown, that the adhesion for hMSCs was best at 46° and for mMSCs at 73°. Again, it was pointed out that a surface of intermediate wettability allowed the best cell adhesion. In a study by Barrias *et al.*, a different trend was found using the same method. In their study, which focussed on adhesion protein adsorption, hMSCs were found to adhere the best for the most hydrophilic composition of the monolayers.[57] In this study however, the substrates were pre-treated for 30 min in the culture medium. The fact that this seems to change the stem cell adhesion completely, indicates that although very similar approaches with only minor changes in procedure can have significant consequences. Another study from Mei *et al.* presents a combinatorial approach, where the effect of roughness, modulus and wettability on hESCs was investigated.[12] They found, that there was a clear influence from wettability towards hESC adhesion. The best adhesion was found for a wettability value of ca. 70° WCA. In all these studies about stem cells, uncharged species were used to investigate the effect of wettability towards the cell behaviour. For neutral surfaces the wettability clearly shows a large influence on stem cell adhesion. Most of these studies do not elaborated much on the issue of stem cell renewal versus stem cell differentiation.

In fact, to our knowledge there are no studies illustrating that wettability can regulate differentiation of stem cells in a direct manner. Nevertheless, differentiation is a highly important aspect of stem cell behaviour, especially in tissue compatibility/integration and tissue regeneration and must therefore be addressed. Furthermore, surface properties need to be extended towards both negatively and positively charged species. A systematic approach of the effect of wettability arising from different chemical species towards stem cells is missing. A study using such an approach, but using other cells, by Arima *et al.* displayed that there are significant differences in stem cell adhesion.[58] Surfaces with the same wettability but different chemical functionalities influenced the stem cell adhesion in a different manner. Even though a trend was shown, pointing out that neither really hydrophilic, nor really hydrophobic surfaces are beneficial for the stem cell adhesion, the wettability alone does not seem to be enough to determine the surface effects on stem cell activity. Since it is now realised that cells adhere to proteins and not to the surface directly, the main aspect for directing stem cell fate is by manipulating the protein layer. Different polar groups can influence proteins differently and can induce denaturation of a protein or alter the orientation. Importantly, the heat inactivated serum added to the culture medium for culturing stem cells might also affect structure of proteins present in the serum. While the effect of protein denaturation on stem cell properties has not yet been properly investigated, orientation effects are known and specific interactions between charges on the protein and the substrate surface will influence the orientation of proteins adsorbing from the serum.[59] The orientation dictates whether the cell surface receptors are able to recognize the individual adhesive proteins and can bind to the corresponding recognition sites.[60] Whereas fibronectin was found to adsorb most strongly to hydrophobic surfaces, vitronectin can easily change its arrangement and adheres also to hydrophilic surfaces.[60] Therefore, investigations involving wettability need to be broadened and should include the full spectrum of available charged surfaces including reversibly charged surfaces, in which the solvent-exposed groups are weakly alkaline (e.g. primary, secondary and tertiary amines) or acidic (carboxylic acids), as well as permanently charged ones, including phosphonic acids and sulphonic acids (anionic; neutral when pH<1.6) or quaternary ammonium groups (cationic).[61]

Stem cells and chemical functionality

As mentioned above, the wettability in most cell related studies is mainly affected by the chemical functionality of the surface. It was observed that chemical functionality of a substrate influences the behaviour of stem cells. Ren and co-workers found that neuronal stem cells (NSCs) behave differently towards surfaces modified with a variety of functional groups.[62] They tested surfaces with –OH, $–NH_2$, $–SO_3H$, –COOH, –SH and $–CH_3$ functionalities. Except for the –OH functionality, all the functionalities were derived from SAMs generated on glass using silanes with different functional solvent-exposed groups. Some of the SAMs were further modified to change the functionality. The –OH functionality was introduced by cleaning the glass slides with piranha solution (H_2SO_4/H_2O_2) and exposing them to deionized water. It was seen, that NSCs have completely different adhesion, proliferation and differentiation behaviour depending on the functionality of the surface. Under physiological conditions, the $–NH_2$ functionalized surfaces are positively charged as $–NH_3^+$ and provided the highest number of adhered viable cell as well as an increase in neural differentiation.[62] The water contact angle of this surface was 58°, which represents an intermediate wettability. Surfaces with a $–SO_3H$ functionality (negatively charged under physiological conditions) provided the best spreading on a single cell level and supported the differentiation into oligodendrocytes.[62] These surfaces were hydrophilic with a contact angle of 19°. NSCs cultured on –COOH (negatively charged under physiological conditions) and –SH surfaces also induced good cell adhesion, but differentiated more into glial cells. Even though these two different surfaces showed similar behaviour, the contact angles were different with 24° (–COOH) and 58° (–SH), which is striking since $–NH_2$ modified surfaces with also a contact angle of 58° displayed different behaviour. The –OH functionalized surfaces induced a weak cell adhesion and provided differentiation into astrocytes. This surface was very hydrophilic with a contact angle of 9°.Compared to other studies, chemical functionalities could here directly be linked to differentiation behaviour of NSCs. Where the wettability of a surface showed to have an effect on the cell adhesion, which is also supported by this study, the chemical functionality affected the differentiation behaviour. In this work it can also be found, that the migration of NSCs seems to follow a similar trend as adhesion in wettability. The highest amount of migrating cells was found on amine functionalised surfaces. Hydroxy- or alkane modified surfaces on the other hand induced low numbers of migrating cells. These findings indicate, that wettability and chemical functionality can influence different behaviours of the cell. This is a further indication for the need of studies, which alter both, wettability and chemical functionality in such a way that strong conclusions can be drawn. Another study, which investigated the effect of surface functionality towards hMSC behaviour, was performed by Curran and co-workers.[63] The surfaces they used were also prepared using glass in combination with functionalized silanes. It was shown, that a $–CH_3$ modified surface maintained the hMSCs phenotype. The –SH and $–NH_2$ modified surfaces supported osteogenic differentiation of hMSCs (Figure 2right), whereas –COOH (Figure 2right) and –OH surfaces supported chondrogenic differentiation. Although, chemical functionality has an effect on wettability, no clear correlation between cell behaviour and wettability was described in this study. Here differentiation appeared to be impacted by the wettability but not by the specific chemistry since –SH and $–NH_2$ have different chemistry but similar wettability and direct the stem cell in the same direction. The same holds for –COOH and –OH, both hydrophilic but different in chemistry and initiating the same differentiation. The chemical composition influences

stem cell behaviour also indirectly through the protein layer. In Figure 3 possible interactions are indicated, which can spontaneously occur at the interface under cell culturing conditions.

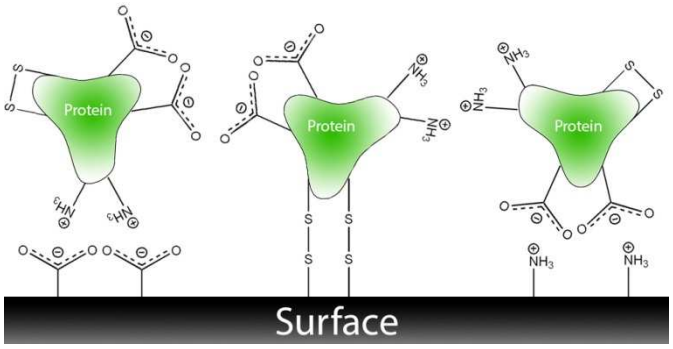
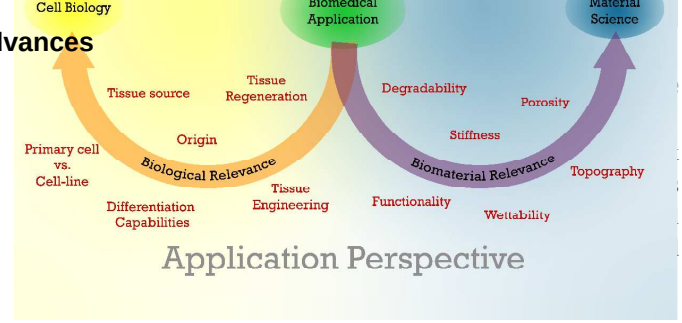


Figure 3: Illustration of a protein binding to the surface. Three different possible interactions are displayed: on the left the surface has acid functionalities, which are deprotonated at neutral pH and have a negative charge. They interact with positive charged moieties of the protein. In the middle a disulphide bridge is displayed, which can be formed from thiols of the surface and the protein respectively. The right side shows also a charge interaction, but for the case, that the surface is positively charged and interacts with negatively charged parts of the protein.

Findings by Anseth *et al.* gave further proof that chemical functionality can direct the fate of hMSCs.[64] In this case, the hMSCs were encapsulated in hydrogels with different functionalities. These hydrogels contained small amounts of different tether molecules, which influence the chemical micro-environment. It was found that the introduction of carboxylic acid functionalities (–COOH) increased the collagen type II expression, which is a chondrogenic marker. Phosphate functionalities ($–OPO(OH)_2$) enhanced the osteogenic differentiation along with elevated expression of osteogenic markers, whereas the hydrophobic *tert*-buthyl functionality ($–C(CH_3)_3$) displayed higher levels of adipogenic markers. These findings illustrate that also in the case of encapsulated stem cells the chemical environment is important for stem cell fate. This influence needs to be investigated, since in the 3D approach the aliphatic modification increased adipogenic markers, whereas in the 2D approach the aliphatic modification did not affect the phenotype of the hMSCs. Also the phosphate modification in 3D led to the same change in phenotype as the amino and thiol modification in 2D.Even in hydrogel systems the protein interactions could play a predominant role, although a more dynamic behaviour seems more likely than the formation of an adhesion layer. This could be a reason, why the hMSCs responded differently in the hydrogel system. On the other hand it could also be that the mechanical properties of the material is responsible for that.

Stem cells and modulus

Stem cells are known to be mechano-sensitive. Numerous studies already investigated the response of stem cells towards the mechanical properties of a biomaterial. The mechanical properties of a material are mostly quantified by measuring the



patterns.[8,65,66] Most studies regarding different elastic moduli have been performed using cross-linked polymers. With those, the modulus can be adjusted by changing the cross-linker density. Previous studies by Engler and co-workers focussed on interactions between MSCs and variably compliant pAAm gels.[4] They showed, that MSCs react to the substrate stiffness with respect to morphology and differentiation capacity. Also a higher collagen type I expression was found with increasing stiffness. In addition, they initiated further research on the role of substrate stiffness and other physicochemical parameters on stem cell fate. The pAAm gels with a variable stiffness influenced adhesion, proliferation and differentiation potential of MSCs. While on more rigid (up to 115 kPa) pAAm, the osteogenic differentiation of MSCs was increased, soft (0.5 – 2 kPa) acrylamide gels directed MSCs to differentiate towards adipocytes. Even though mechanical stimuli are recognized to be highly important, opposite MSCs differentiation potential between pAAm and polydimethylsiloxane(PDMS) with the same stiffness have been found indicating that a single parameter such as stiffness alone does not provide the full scope.[7] In addition, following the data by Evans *et al.*, culturing of ESCs on polydimethylsiloxane (PDMS) with an increasing stiffness (0.041 kPa-2.7 MPa), and covalently linked type I collagen, led to both enhanced proliferation and differentiation of ESCs.[6] In contrast to ESCs, spreading and differentiation of MSCs was not regulated by PDMS elasticity. These opposite results between PDMS could result from the fact that the materials stiffness was measured only before collagen coating and not after. Therefore, the stiffness data may not be completely comparable. Furthermore, the simple difference in the biomaterial type could result in a conformational change of the protein layer. This in turn could lead to incorrect conclusions about the stiffness effect on MSCs differentiation.

Healy and co-workers investigated the effect of the elastic modulus of a 2D culture substrate towards differentiation of neural stem cells (NSCs) was investigated.[67] By using RGD (RGD motif consisting of 3 amino-acids Arg-Gly-Asp; a cell adhesive peptide)-modified polymer networks with different moduli, they found that NSCs are able to self-renew and differentiate for gels with a minimum stiffness of 100 Pa. In addition, substrates with an elastic modulus of ~500 Pa were optimal for a neural differentiation. Banerjee *et al.* found a clear trend for adhesion and proliferation of encapsulated NSCs towards substrate stiffness.[68] Here, it was observed that in a 3D microenvironment, adhesion and proliferation is best for the substrate with the lowest stiffness of 183 Pa. Even though the optimum point from the study of Healy *et al.* was not measured, both studies displayed different results for the adhesion. It has to be considered that one study was performed in a 2D and the

other is a 3D setting. In addition, two different polymeric networks were used, which can significantly influence the adhesion behaviour as pointed out in the sections above. It has to be noted that determining the stiffness is not a trivial task. The stiffness is often determined either via macroscopic indentation or by atomic force microscopy (AFM). Macroscopic indentation measures the bulk while AFM measures the surface stiffness. The question is of course what exactly the cell will respond to and therefore, which value is the correct measure for the stiffness especially since Discher and co-workers found that the stiffness depends on the thickness of the layer under investigation so probably it is be a combined contribution of both the top layer and substrate beneath.[36] Different studies have been reported which applied either the one or the other approach for measuring the stiffness. Comparing results from such studies will therefore be difficult and this may lead to contradicting results. Also when dealing with hydrogels, the protein adhesion layer might not be confined to the surface alone but also penetrate into the hydrogel network. This was also suggested recently explaining the potential differences in mechanical stimuli between pAAm gels and PDMS with the same mechanical properties.[7] It is therefore important to systematically measure all properties for both bulk and surface before as well as after applying any protein adhesion layers (as indicated in **Figure 1**). Notably, Choi *et al.* have measured pAAm and did not find significant difference in stiffness before and after coating with collagen type I when culturing HSCs.[69] Applying three different concentrations of the rat collagen type I (1 ng/ml, 40 μg/ml and 100 μg/ml) for coating of a constant stiffness PAAm196 kPa (±54kPa), resulted in a decreased HSCs viability and cell spreading observed with a decreasing collagen concentration. These results may suggest that rather than the biomaterial stiffness, the collagen concentration had a large impact on HSC behaviour. Additionally, they used acrylamide gels ranging from 0.7-196 kPa and only verified the before and after results for the 196 kPa gel while the strongest effects can be expected for the gels of lower stiffness. Especially, since collagen as a gel has been measured to have a Young's modulus ranging from 1-28kPa depending on the concentration and individual collagen fibrils have a much higher modulus in the range of 5-11 GPa.[70,71] Therefore one expects a change upon applying a coating affecting the mechanical properties for the better or worse but affecting nonetheless.

Materials used as stem cell scaffolds

There is a continuous search for finding the most optimal material for harbouring stem cells as it should be able to influence, stimulate and direct stem cells towards the desired fate at interfaces. Such an optimal material could be applied in tissue engineering and regenerative medicine but also should have a strong use as a surface of medical implants in order to facilitate tissue integration or minimizing fibrosis. On many occasions it seems that a random collection of materials (mainly polymers) have been tested with respect to stem cell adhesion and differentiation performance. Of course some studies are identifying the possibilities of most frequently used biomaterials as a competitive alternative to the commonly used 2D TCP and some of them supported proliferation of HSCs even better than TCP.[9] In that study it was also observed that during a long-term HSCs culture some of the used polymers including Resomer® and PCL (polycaprolactone) were more dependent on cytokine addition in order to maintain the growth of HSCs than other materials. Therefore, a spatial structure of biomaterials in suboptimal liquid culture conditions may lead to the unpredictable behaviour of stem cells particularly in the long-term settings. Consequently, it is essential to choose appropriate substrates with a stable, non-degradable structure in the chosen experimental *in vitro* long-term conditions. In addition, this long-term parameter of stem cell culture with a biomaterial remains crucial since the stem cell differentiation protocols include fourteen or more days of culturing.

In addition to the more randomized studies of using materials for stem cell studies, also much more methodological approaches have been undertaken. Recent studies include applying combinatorial biomaterial arrays with variable cross-linking density and polarity showed that the optimal growth of ESCs was supported by biomaterials of a modest polarity (65°<WCA<80°) composed of monomers which had more than one acrylate functionality, influencing the internal cross-linking.[12] Furthermore, many of the tested hydrophilic polymers were swollen due to hydration and therefore created a surface stiffness less than 0.2 GPa, which strongly affected ESC growth. Remarkably, the moderate polarity was optimal for ESCs proliferation over a wide stiffness range up to more than 0.2 GPa, indicating that ESC growth can be regulated not only by the stiffness of a synthetic substrate, but even more by its polarity. This approach provides excellent and tremendous amounts of insights, but the inclusion of charged species and a more clear rationale behind the chosen functionalities would provide even more insights on the most important contributing parameters. The charge of a hydrogel is important as was shown with respect to the response of pluripotent stem cells. A positively charged hydrogel such as DMAEMA ((2-dimethylamino) ethyl methacrylate)) containing cationic polymeric groups had a negative effect on ESCs adhesion,[72] as was exemplified by a significant decrease in expression of E-cadherin, found previously to be implicated in embryonic stem cell adhesion.[73] In contrast, a negatively charged PNaAMPS (poly-(2-acrylamido-2-methyl-propane sulfonic acid sodium salt) hydrogel allowed for the highest and also long-term expansion of iPSCs in a feeder-free *in vitro* culture as compared to a commonly applied gelatin-coated polystyrene.[74]

Effects of stem cell type towards materials

It has been established that different surface conditions such as chemical modifications, mechanical properties and topography affect the behaviour of stem cells, albeit chemistry in an indirect fashion.[12,75–77] It can be expected though that stem cells of different origin in terms of both development and tissue type will behave differently towards the same surface or scaffold

properties. In many of the reported investigations different stem cell types were used, as well as different cell lines and even stem cells from different species. Stem cells will adapt to environmental changes and while the source and the scaffold remains the same still stem cells can respond differently under slightly varying conditions such as the culture passage, culturing history and culturing conditions. While in the previous section stem cell response of the same stem cell type but towards materials with similar properties has been discussed, here we discuss the use of identical materials but combined with different origin stem cells. ESCs, MSCs, HSCs and IPSCs have all been used on numerous occasions since they all have important potential therapeutic applications. However, there is never an extensive comparison and also each stem cell from the same lineage can have different origin with respect to where they reside e.g. MSCs can be derived from various sources such as bone marrow (BM-MSC), fat/adipose tissue (AT-MSC), amniotic fluid, or umbilical cord tissue.[20]It was already stated that culture media and defined culturing conditions are not trivial issues let alone the complexity of stem cell responses from different lineages or same lineage but different origin to a controlled interface.[19]

Deviating response of stem cells of different origin

Stem cells as mentioned above respond to surfaces via an interplay of ECM mechanical properties via trans-membrane receptors acting upon the actin-myosin cytoskeletal reorganization and hence stem cell shape and behaviour. While various surface receptors for e.g. MSCs, have been identified, there is deviation within the composition of these which is attributed to culturing methods and/or differentiation stage as well as solution directed stimuli.[20,78] This alteration in surface receptor composition would of course also be of significance for the physicochemical response of the stem cell.

For long times MSCs have been isolated from bone marrow and considered as highly promising in tissue engineering but also for regenerative medicine.[3,20] In regenerative medicine, MSCs regulate the processes for tissue repair rather than taking part in the actual tissue formation and due to the non-immunogenic character it is also interesting to target not only allogeneic MSCs but also investigate xenogeneic MSC use.[79] Many different tissues of different animals have been studied and used for isolating MSCs but the question to be addressed is whether MSCs from different organisms or stemming from different tissues or cultured under diverse conditions behave the same. It has been found that BM-MSCs and AT-MSCs do indeed not behave the same with respect to differentiation capabilities and similar with respect to various regenerative capabilities for certain diseases.[48,80–86] Many studies have been performed in order to elucidate variations in stem cell responses including MSCs. It was observed and criticized that indeed already many of the basic conditions for culturing and maintaining potency and self-renewal of stem cells need to be taken into account. It was shown that amongst others basal nutrients, growth factor and cytokine compositions, stem cell density all influence both the proliferation and multilineage differentiation of MSCs profoundly and that donor variation exists.[19,78]

Deviating response of stem cells at material interfaces

Although efforts towards identifying the difference in response of stem cells of different developmental origin, derived from different tissue sources and even donor variations have been made, only few studies have linked this issue to interactions with materials studies on this issue are emerging, however, mostly focusing on comparisons between the BM-MSC and AT-MSC since these are therapeutically and clinically most relevant. A study by Voelcker and co-workers combined the use of rat BM-MSC, human BM-MSC and human AT-MSC interacting with the same interface under equal conditions.[87] In this study the most basic cellular responses (attachment and spreading) towards a porous gradient silicon substrate were investigated.[87] It was observed that rat BM-MSCs went from about 600μm^2 cell spreading area at low surface solid fraction (large surface pore diameter) to 6000μm^2 cell spreading area when reaching the other end of the gradient with a high surface solid fraction (small surface pore diameter). Human BM-MSCs under the same conditions displayed a much smaller difference namely 1200$\mu m^2$and 2200μm^2, respectively. The AT-MSC gave values of 3000$\mu m^2$and 5200μm^2. Although, the spreading was different between rat- and human BM-MSCs, the shape was very similar both displaying a more rounded shape as compared to the human AT-MSC which was much more elongated and spindle-like. The next step should include the differentiation capability and efficiency, but these data strongly indicate the importance of these comparative studies.

In many other studies, especially stem cells combined with different mechanical stimuli exerted by the material interface, differentiation is one of the main aspects investigated but never include a similar approach as shown above. While one recent study by Li *et al.* does provide the comparison between rat BM-MSC and rat AT-MSC,[88] all other investigations use only one MSC type. Although, many use rat or human BM-MSCs, proper comparison between studies is very difficult since as stated before, culturing conditions can already affect stem cell behaviour. This is then also combined with specific material properties which often cannot be perfectly duplicated or have been modified slightly therewith implementing more uncertainties. The difference in differentiation capabilities between BM-MSCs and AT-MSCs was already recognized in conventional *in vitro* experiments and can therefore also be expected to have implications when mechanical stimuli are applied by using substrates of different mechanical properties but similar chemistry as was proven by Li *et al.*.[88]By using PDMS of different cross-linking density and hence different stiffness, it was identified that BM-MSCs are more prone to differentiate towards osteoblast lineage than AT-MSCs but only under specific conditions. On stiffer substrates ALP (alkaline phosphatase) was more expressed in BM-MSCs as well as collagen type I and osteocalcin. On soft substrates however, even though expression levels of these proteins were very low for both BM-MSC and AT-MSC, the difference was much less

pronounced indicating that both MSC types respond very differently towards the same mechanical stimulus exerted by the material.[88]

Studies on mechanical effects on stem cell differentiation using either pAAm hydrogels or PDMS substrates (or both) using one type of stem cell have been published. In most cases, human BM-MSCs[7,89–94] or rat BM-MSCs[88,95] were used and occasionally ESCs,[6,96] umbilical cord stem cells (UCSCs)[97] or hematopoietic stem and progenitor cells (HSPCs)[69]. The outcomes of these investigation are difficult if not impossible to compare due to small changes in experimental setups and different levels of analysis. One of these differences is layer thickness, when hydrogels are used such as pAAms. Buxboim *et al.* identified that the layer thickness had a significant influence on the macroscopic behaviour of human BM-MSCs. Cell spreading areas were determined and the spreading area decreased (~2250μm^2 to ~650μm^2) with increasing the layer thickness (500nm to 100μm) of a 1 kPa pAAm gel on glass. The thinner the gel layer the more of the underlying substrate the cell "feels" and hence the gel is considered to be stiffer than the actual prepared gel layer meaning that cell spreading increases with increasing matrix stiffness. This corresponded very well with their previous studies. In retrospect, other results can be interpreted much more clearly knowing these facts. Trappmann *et al.* found much higher cell spreading areas at the same stiffness indicating that the layer thickness was most likely much thinner as compared to the layers used by Engler *et al.* and Buxboim *et al.*.[7,89,94] Both intrinsic stem cell properties and conditions of the stem cell niche as defined by culture conditions and material properties dictate the differentiation outcome. The intrinsic cellular stiffness (Figure 4) is a significant part of this equation. For example, ESCs cultured on 2D TCP have been shown to be at least two-fold stiffer than MSCs[98]. The reported differences in the elastic properties between embryonic ESCs and adult MSCs might indicate the presence of developmentally defined mechanical forces, to which both of these stem cell types are exposed *in vivo* and/or for the presence of differences arising from a distinct developmental origin (Figure 4). When looking at the different stem cells, they have intrinsically different stiffness, differentiate into different tissues based on mechanical cues exerted by the materials/substrate as well as the wettability of the material. The intrinsic cellular properties combined with external influences such as the materials on which the stem cells are cultured, illustrates the delicate balance between the two (Figure 4). Evans *et al.* illustrated that ESCs cultured on collagen type I coated PDMS with an increasing stiffness (0.041 kPa-2.7 MPa) led to both enhanced proliferation and differentiation of ESCs.[6] In contrast to ESCs, spreading and differentiation of MSCs was not regulated by the PDMS elasticity (0.1 kPa–2.3 MPa), as described by Trappmann *et al.*.[7]These opposite results between ESCs and MSCs imply that stem cell-specific differences regarding the developmental origin exist which is even present among MSCs as was shown for BM-MSC and AT-MSC with respect to osteogenic differentiation. Even though the potential of efficient differentiation of AT-MSCs remains, when treated correctly by first applying a pre-chondrogenic induction step before targeting the osteogenic lineage, this difference in potential can be circumvented[48]. In a very elaborate study Musah *et al.* compared the behaviour of 10 different stem cell lines (5 human ESCs, 5 iPSCs) interacting with a set of glycosaminoglycan-binding hydrogels possessing different mechanical properties.[99]Although only highlighting one cell type specifically, all cell lines maintained the expression of pluripotency markers (Oct4, SSEA-4) when expanding on the stiffer gels. However, it would also be interesting to investigate the overall numbers concerning proliferation, cell spreading and differentiation capabilities not only for the stiffer gel-layer which maintained pluripotency but also for the softer ones.

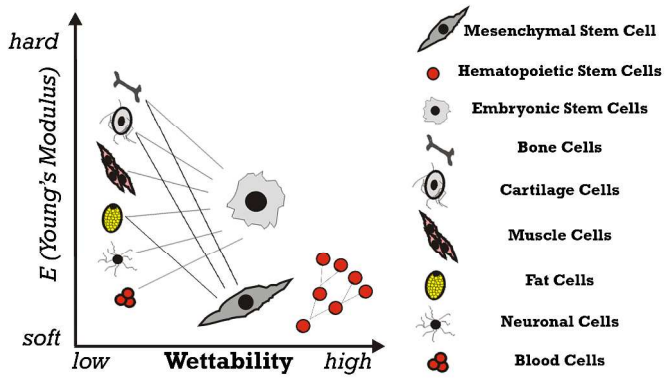


Figure 4: Stem cells of different origin and their physicochemical preferences. Stem cells of different origin may also be distinguished by their physicochemical preferences for a given substrate. Although ESCs are stiffer than MSCs ,[63], which is shown here as the function of E, representing the stiffness, both of these stem cells prefer to grow on substrates with a moderate wettability. In contrast, HSCs being the softest type among stem cells presented here, were found to have the optimal growth on hydrophilic substrates. Further, in the context of stiffness each of these stem cells produce progeny depending on the stiffness range: 1) multipotent HSCs giving rise to all different types of blood cells with specific function but all representing rather uniform stiffness; 2) MSCs can differentiate into bone, cartilage and fat, which as progeny are representing much higher stiffness range compared to HSCs and 3) ESCs being the most potent (pluripotent) from the presented stem cells, generate the progeny of the largest stiffness range.

Apart from the stem cell type and culture conditions, stem cell density has been reported to be crucial as well in directing differentiation. Rödling *et al.*[100] reported that the fate of stem cells growing on synthetic or naturally-derived biomaterials depends on cell density. On 2D feeder-free cultures and hyaluronan (HA)-based hydrogels the self-renewal of ESCs was maintained at a relatively high cell density. In contrast to ESCs, a too high plating density of HSCs (20.000 cells/ml) in 2D TCP resulted in a decreased proliferation of stem cells compared to a low seeding density (5.000 cells/ml),[100] which may result from a relatively fast accumulation of inhibitory factors produced by expanded HSCs as demonstrated by Csaszar *et al.*.[101] Therefore, maintaining self-renewal of both ESCs and HSCs on a synthetic substrate also depends on the stem cell density. It is then apparent that regulation of stem cell fate decisions by a biomaterial may be altered by improper density of stem cells when cultured in 3D substrates or

improper so called confluency when regarding cell density on 2D substrates. Moreover, examination of the elastic properties of ESCs and MSCs has shown that based on 2D TCP cultures of single ESCs and MSCs, ESCs were at least two-fold stiffer than MSCs,[63] suggesting that by analysing the impact of elasticity one should examine a collective stiffness, which includes stiffness of the biomaterial, protein / ECM coating and applied stem cells. All the issues raised above indicate that a well-controlled experimental setup needs to include multiple aspects including the proper stem cells, culture conditions and material selection as well as verification of determined parameters after every alteration or addition.

Possible Memory Effect of Stem Cells

The broad and extensive application of 2D liquid *in vitro* cultures using traditional tissue culture polystyrene (TCP) vessels is the standard for culturing cells and also frequently used for various stem cells.[78,102] MSCs are described as stem cells typically adhering to surfaces, often in combination with a pre-coating of ECM protein layers.[35] In contrast to MSCs, HSCs are known as non-adherent stem cells although also non-adhering MSCs have been identified.[103] So far they have been maintained and expanded *ex vivo* only to a limited extent in so called 'co-culture' systems by growing them on top of a mitotically-inactivated feeder cell layer.[101] Similarly, current culture methods for ESCs and iPSCs also include feeder-dependent growth although successful attempts have been made to prepare feeder-free approaches.[19,104–107] It is realised, however, that TCP probably is not appropriate and specific enough for optimal culturing of stem cell with respect to both self-renewal and differentiation. As substrate mechanics play a key role in differentiation capacity, the reported biased osteogenic differentiation potential of MSCs, particularly at a high initial seeding cell density should not be surprising[108], especially since the stiffness of polystyrene (PS) is in a range of 3-3.5 GPa,[109,110] which reaches the upper limit of the physiological stiffness of fully mineralized bone. This affects differentiation of ESCs much less since these are generally cultured in a spheroid culture, the so called embryoid bodies (EBs) and differentiation in TCP-based cultures is mainly directed by the 3D nature of the EBs (reviewed by Chai and Leong [111]).

The culture history of cells is known to influence its properties. This has been observed for murine BM-MSCs of which the differentiation capacity is influenced by both age of the donor and by the passage number.[47]The decreased capacity with increasing donor age or culture time may be due to genetic changes but it may also indicate the presence of a form of age memory. That stem cells possess stored memory has recently been shown by Yang *et al.*[110] who cultured MSCs on soft (~2 kPa) PEG (poly(ethylene glycol)) after a long-term (10 days) TCP-based culture which resulted in a significantly enhanced osteogenic differentiation. In addition, MSCs were not able to differentiate into the adipogenic lineage even in the presence of bipotential osteogenic/adipogenic differentiation culture medium, which strongly suggests that MSCs possess the irreversible mechanical memory of previous culture conditions.[110]Thus, an extended culture time on a very stiff surface providing a mechanical dosing/stimulus, may irreversibly impair the multilineage differentiation potential of MSCs. This study offers "food for thought" with respect to the actual response of the stem cells we are investigating, certainly if we have to include the entire history of the cells. Although the study of Yang *et al.* indicates that the complexity of cell-material interactions run much deeper than realized so far, it was not emphasized or realized that the exposure time of MSCs on TCP actually was much longer than 10 days. Although for all experiments the first passage (P1) of MSCs was used, the isolation of these stem cells required a preferential adhesion to TCP for at least 2 weeks. Therefore, there might be an additive effect of TCP on the increased osteogenic potential and impaired multilineage differentiation potential of MSCs. Another emerging important question, which needs to be addressed is if *in situ* hardening would reverse the effect of *in situ* softening on multilineage differentiation, further strengthening the concept of the presence of a mechanical memory of MSCs. Another valid question would then also be whether a prior prolonged exposure to TCP also affected the growth of MSCs as observed for ageing and passage number effects observed in the study by Kretlow *et al.* mentioned earlier.[47]

Towards designing biomaterials with a greater impact: Conclusions and Perspectives

The use of stem cell potential in terms of self-renewal and multilineage differentiation still remains limited in tissue engineering and regenerative medicine applications. One of the main reasons is an iterative and randomized process of studying biomaterials as discussed in this review. There also is a lack of pre-selection procedures for biomaterials specific for a particular applied stem cell.[9,10] In addition, a selection of the appropriate adhesion proteins, material wettability, chemistry, mechanics and topography appears to be necessary. As a crucial example pAAm can be taken, a polymer that is broadly applied to study (stem) cell behaviour and originally used in electrophoresis.[112] Leaching of acrylamide monomers after solidifying has been found to be cytotoxic to cells.[113,114] Another potential limitation of acrylamide for both regenerative medicine and tissue engineering purposes, is its small elasticity range of 1-100 kPa,[4,115] which is lower than the elasticity of many tissues within the human body. Higher moduli cannot be achieved with the same material, making higher values difficult to compare due to a change in scaffold material.[116] Therefore, the choice of biomaterial for stem cell-based studies should be performed very carefully and the range of parameters which can be targeted with the material should be carefully considered. One of the main aspects still missing in many of the studies is a unified approach to investigate responses of stem cells and compare behaviours. This does not only concern the cell biology part but also the materials science and (physico-)chemical aspect. A unified approach offers a more general

consensus and quicker assimilation of knowledge to identify the true and most important parameters as well as a better comparison between experiments performed between research groups. Figure 1 represents the most important parameters for such a unified approach which takes aspects as cytotoxicity/compatibility, protein adhesion layers, proliferation and differentiation into account from the cell biological approach which is combined with a full scope of parameters for the materials approach in which the mechanical properties, chemistry and topography is carefully identified for both the bulk materials and the surface, keeping the initial application or specific research question in mind as the central theme. By approaching both these topics in this highly structured fashion, the most appropriate biomaterial for the desired application should be reached quicker and in a more reliable way.

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Bibliography

1. B. M. Holzapfel, J. C. Reichert, J.-T. Schantz, U. Gbureck, L. Rackwitz, U. Nöth, F. Jakob, M. Rudert, J. Groll, and D. W. Hutmacher, *Adv. Drug Deliv. Rev.*, 2013, **65**, 581–603.
2. C. Vacanti, *J. Cell. Mol. Med.*, 2006, **1**, 569–576.
3. A. I. Caplan, *J. Cell. Physiol.*, 2007, **213**, 341–347.
4. A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, *Cell*, 2006, **126**, 677–89.
5. L. MacQueen, Y. Sun, and C. A. Simmons, *J. R. Soc. Interface*, 2013, **10**, 20130179.
6. N. D. Evans, C. Minelli, E. Gentleman, V. LaPointe, S. N. Patankar, M. Kallivretaki, X. Chen, C. J. Roberts, and M. M. Stevens, *Eur. Cell. Mater.*, 2009, **18**, 1–13.
7. B. Trappmann, J. E. Gautrot, J. T. Connelly, D. G. T. Strange, Y. Li, M. L. Oyen, M. a Cohen Stuart, H. Boehm, B. Li, V. Vogel, J. P. Spatz, F. M. Watt, and W. T. S. Huck, *Nat. Mater.*, 2012, **11**, 642–9.
8. O. Z. Fisher, A. Khademhosseini, R. Langer, and N. a Peppas, *Acc. Chem. Res.*, 2010, **43**, 419–28.
9. M. V. Ferreira, N. Labude, D. Piroth, W. Jahnen-Dechent, R. Knüchel, T. Hieronymus, M. Zenke, and S. Neuss, *J. Mater. Sci. Mater. Med.*, 2012, **23**, 109–16.
10. M. S. V. Ferreira, W. Jahnen-Dechent, N. Labude, M. Bovi, T. Hieronymus, M. Zenke, R. K. Schneider, S. Neuss, and S. Neurs, *Biomaterials*, 2012, **33**, 6987–97.
11. P. Tsimbouri, N. Gadegaard, K. Burgess, K. White, P. Reynolds, P. Herzyk, R. Oreffo, and M. J. Dalby, *J. Cell. Biochem.*, 2014, **115**, 380–90.
12. Y. Mei, K. Saha, S. R. Bogatyrev, J. Yang, A. L. Hook, Z. I. Kalcioglu, S.-W. Cho, M. Mitalipova, N. Pyzocha, F. Rojas, K. J. Van Vliet, M. C. Davies, M. R. Alexander, R. Langer, R. Jaenisch, and D. G. Anderson, *Nat. Mater.*, 2010, **9**, 768–78.
13. R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, and C. S. Chen, *Dev. Cell*, 2004, **6**, 483–95.
14. J. Genzer, *Annu. Rev. Mater. Res.*, 2012, **42**, 435–468.
15. J. Genzer and R. R. Bhat, *Langmuir*, 2008, **24**, 2294–2317.
16. A. Mierczynska, A. Michelmore, A. Tripathi, R. V. Goreham, R. Sedev, and K. Vasilev, *Soft Matter*, 2012, **8**, 8399.
17. D.-H. Kim, K. Han, K. Gupta, K. W. Kwon, K.-Y. Suh, and A. Levchenko, *Biomaterials*, 2009, **30**, 5433–44.
18. R. J. Wade and J. A. Burdick, *Mater. Today*, 2012, **15**, 454–459.
19. F. Mannello and G. A. Tonti, *Stem Cells*, 2007, **25**, 1603–9.
20. M. Kassem, M. Kristiansen, and B. M. Abdallah, *Basic Clin. Pharmacol. Toxicol.*, 2004, **95**, 209–14.
21. H. Rippon and A. Bishop, *Cell Prolif.*, 2004, 23–34.
22. E. Dzierzak, *Immunol. Rev.*, 2002, **187**, 126–38.
23. I. Chambers and A. Smith, *Oncogene*, 2004, **23**, 7150–60.
24. G. Keller, *Genes Dev.*, 2005, **19**, 1129–55.
25. H. Inoue, N. Nagata, H. Kurokawa, and S. Yamanaka, *EMBO J.*, 2014, **33**, 409–17.
26. K. Takahashi and S. Yamanaka, *Development*, 2013, **140**, 2457–61.
27. K. Takahashi and S. Yamanaka, *Cell*, 2006, **126**, 663–76.
28. R. Jaenisch and R. Young, *Cell*, 2008, **132**, 567–82.
29. M. Krampera, G. Pizzolo, G. Aprili, and M. Franchini, *Bone*, 2006, **39**, 678–83.
30. S. C. Mendes, C. Robin, and E. Dzierzak, *Development*, 2005, **132**, 1127–36.
31. B. J. H. Jansen, C. Gilissen, H. Roelofs, A. Schaap-Oziemlak, J. a Veltman, R. A. P. Raymakers, J. H. Jansen, G. Kögler, C. G. Figdor, R. Torensma, and G. J. Adema, *Stem Cells Dev.*, 2010, **19**, 481–90.
32. S. Méndez-Ferrer, T. V Michurina, F. Ferraro, A. R. Mazloom, B. D. Macarthur, S. A. Lira, D. T. Scadden, A. Ma'ayan, G. N. Enikolopov, and P. S. Frenette, *Nature*, 2010, **466**, 829–34.
33. A. Wilson, G. M. Oser, M. Jaworski, W. E. Blanco-Bose, E. Laurenti, C. Adolphe, M. a Essers, H. R. Macdonald, and A. Trumpp, *Ann. N. Y. Acad. Sci.*, 2007, **1106**, 64–75.
34. M. a Bucaro, Y. Vasquez, B. D. Hatton, and J. Aizenberg, *ACS Nano*, 2012, **6**, 6222–30.
35. A. Higuchi, Q.-D. Ling, Y. Chang, S.-T. Hsu, and A. Umezawa, *Chem. Rev.*, 2013, **113**, 3297–328.
36. A. Buxboim, K. Rajagopal, A. E. X. Brown, and D. E. Discher, *J. Phys. Condens. Matter*, 2010, **22**, 194116.
37. D. DeSimone and R. Mecham, *Extracellular Matrix in Development*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013.
38. B. Geiger, J. P. Spatz, and A. D. Bershadsky, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 21–33.
39. M. Guvendiren and J. A. Burdick, *Nat. Commun.*, 2012, **3**, 792.
40. W. L. K. Chen and C. a Simmons, *Adv. Drug Deliv. Rev.*, 2011, **63**, 269–76.
41. Y. Tamada and Y. Ikada, *J. Colloid Interface Sci.*, 1993, **155**, 334–339.
42. J. H. Lee and H. B. Lee, *J. Biomed. Mater. Res.*, 1998, **41**, 304–11.
43. M. Rabe, D. Verdes, and S. Seeger, *Adv. Colloid Interface Sci.*, 2011, **162**, 87–106.
44. M. Mendicino, A. M. Bailey, K. Wonnacott, R. K. Puri, and S. R. Bauer, *Cell Stem Cell*, 2014, **14**, 141–5.
45. J. Brower, M. Student, S. Blumberg, P. Fellow, E. Carroll, I. Pastar, H. Brem, and W. Chen, *Adv. Ski. Wound Care*, 2012, **24**, 524–532.
46. A. F. Steinert, L. Rackwitz, F. Gilbert, U. Nöth, and R. S. Tuan, *Stem Cells Transl. Med.*, 2012, **1**, 237–47.

47. J. D. Kretlow, Y.-Q. Jin, W. Liu, W. J. Zhang, T.-H. Hong, G. Zhou, L. S. Baggett, A. G. Mikos, and Y. Cao, *BMC Cell Biol.*, 2008, **9**, 60.
48. J. Brocher, P. Janicki, P. Voltz, E. Seebach, E. Neumann, U. Mueller-Ladner, and W. Richter, *Stem Cell Res.*, 2013, **11**, 1393–406.
49. A. Higuchi, Q. D. Ling, Y. Chang, S. T. Hsu, and A. Umezawa, *Chem Rev*, 2013, **113**, 3297–3328.
50. M. M. Stevens and J. H. George, *Science*, 2005, **310**, 1135–8.
51. E. S. Place, N. D. Evans, and M. M. Stevens, *Nat. Mater.*, 2009, **8**, 457–70.
52. J. H. Lee, H. W. Jung, I. K. Kang, and H. B. Lee, *Biomaterials*, 1994, **15**, 705–11.
53. J. M. Rodríguez Patino, M. R. Rodríguez Niño, and C. Carrera Sánchez, *Curr. Opin. Colloid Interface Sci.*, 2007, **12**, 187–195.
54. J. L. Zhai, L. Day, M.-I. Aguilar, and T. J. Wooster, *Curr. Opin. Colloid Interface Sci.*, 2013, **18**, 257–271.
55. Y. N. Shin, B. S. Kim, H. H. Ahn, J. H. Lee, K. S. Kim, J. Y. Lee, M. S. Kim, G. Khang, and H. B. Lee, *Appl. Surf. Sci.*, 2008, **255**, 293–296.
56. L. Hao, H. Yang, C. Du, X. Fu, N. Zhao, S. Xu, F. Cui, C. Mao, and Y. Wang, *J. Mater. Chem. B*, 2014.
57. C. C. Barrias, M. C. L. Martins, G. Almeida-Porada, M. a Barbosa, and P. L. Granja, *Biomaterials*, 2009, **30**, 307–16.
58. Y. Arima and H. Iwata, *Biomaterials*, 2007, **28**, 3074–82.
59. M. I. Fitchmun, J. Falk-Marzillier, E. Marshall, G. Cruz, J. C. Jones, and V. Quaranta, *Anal. Biochem.*, 1998, **265**, 1–7.
60. N. Faucheux, R. Schweiss, K. Lützow, C. Werner, and T. Groth, *Biomaterials*, 2004, **25**, 2721–30.
61. A. Ulman, *Chem. Rev.*, 1996, **96**, 1533–1554.
62. Y.-J. Ren, H. Zhang, H. Huang, X.-M. Wang, Z.-Y. Zhou, F.-Z. Cui, and Y.-H. An, *Biomaterials*, 2009, **30**, 1036–44.
63. J. M. Curran, R. Chen, and J. a Hunt, *Biomaterials*, 2006, **27**, 4783–93.
64. D. S. W. Benoit, M. P. Schwartz, A. R. Durney, and K. S. Anseth, *Nat. Mater.*, 2008, **7**, 816–23.
65. E. Fuchs, T. Tumbar, and G. Guasch, *Cell*, 2004, **116**, 769–78.
66. D. E. Discher, D. J. Mooney, and P. W. Zandstra, *Science*, 2009, **324**, 1673–7.
67. K. Saha, A. J. Keung, E. F. Irwin, Y. Li, L. Little, D. V Schaffer, and K. E. Healy, *Biophys. J.*, 2008, **95**, 4426–38.
68. A. Banerjee, M. Arha, S. Choudhary, R. S. Ashton, S. R. Bhatia, D. V Schaffer, and R. S. Kane, *Biomaterials*, 2009, **30**, 4695–9.
69. J. S. Choi and B. a C. Harley, *Biomaterials*, 2012, **33**, 4460–8.
70. M. P. E. Wenger, L. Bozec, M. a Horton, and P. Mesquida, *Biophys. J.*, 2007, **93**, 1255–63.
71. M. C. Lopez-garcia, D. J. Beebe, and W. C. Crone, 2009.
72. J. Kroupová, D. Horák, J. Pacherník, P. Dvorák, and M. Slouf, *J. Biomed. Mater. Res. B. Appl. Biomater.*, 2006, **76**, 315–25.
73. M. A. Conti, S. Even-Ram, C. Liu, K. M. Yamada, and R. S. Adelstein, *J. Biol. Chem.*, 2004, **279**, 41263–6.
74. J. J. Yang, J. F. Liu, T. Kurokawa, K. Kitada, and J. P. Gong, *J. Tissue Eng. Regen. Med.*, 2012, doi: 10.1002/term.1640.
75. C. J. Bettinger, R. Langer, and J. T. Borenstein, *Angew. Chem. Int. Ed.*, 2009, **48**, 5406–15.
76. R. Ayala, C. Zhang, D. Yang, Y. Hwang, A. Aung, S. S. Shroff, F. T. Arce, R. Lal, G. Arya, and S. Varghese, *Biomaterials*, 2011, **32**, 3700–11.
77. D. E. Discher, P. Janmey, and Y.-L. Wang, *Science*, 2005, **310**, 1139–43.
78. M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig, D. R. Marshak, *Science*, 1999, **284**, 143–147.
79. P. Niemeyer, T. S. Schönberger, J. Hahn, P. Kasten, J. Fellenberg, N. Suedkamp, A. T. Mehlhorn, S. Milz, and S. Pearce, *Tissue Eng. Part A*, 2010, **16**, 33–43.
80. P. A. Zuk, M. Zhu, H. Mizuno, J. Huang, J. W. Futrell, a J. Katz, P. Benhaim, H. P. Lorenz, and M. H. Hedrick, *Tissue Eng.*, 2001, **7**, 211–28.
81. M. Strioga, S. Viswanathan, A. Darinskas, O. Slaby, and J. Michalek, *Stem Cells Dev.*, 2012, **21**, 2724–52.
82. M. Roemeling-van Rhijn, M. Khairoun, S. S. Korevaar, E. Lievers, D. G. Leuning, J. N. Ijzermans, M. G. Betjes, P. G. Genever, C. van Kooten, H. J. de Fijter, T. J. Rabelink, C. C. Baan, W. Weimar, H. Roelofs, M. J. Hoogduijn, and M. E. Reinders, *J. Stem Cell Res. Ther.*, 2013, 20780.
83. C. Pendleton, Q. Li, D. a Chesler, K. Yuan, H. Guerrero-Cazares, and A. Quinones-Hinojosa, *PLoS One*, 2013, **8**, e58198.
84. J. S. Elman, M. Li, F. Wang, J. M. Gimble, and B. Parekkadan, *J. Inflamm.*, 2014, **11**, 1.
85. W. Zhang, X. Zhang, S. Wang, L. Xu, M. Zhang, G. Wang, Y. Jin, and X. Jiang, *J. Dent. Res.*, 2013, **92**, 1136–41.
86. P. Niemeyer, K. Fechner, S. Milz, W. Richter, N. P. Suedkamp, A. T. Mehlhorn, S. Pearce, and P. Kasten, *Biomaterials*, 2010, **31**, 3572–9.
87. P.-Y. Wang, L. R. Clements, H. Thissen, S.-C. Hung, N.-C. Cheng, W.-B. Tsai, and N. H. Voelcker, *RSC Adv.*, 2012, **2**, 12857.
88. X. Li, Y. Huang, L. Zheng, H. Liu, X. Niu, J. Huang, F. Zhao, and Y. Fan, *J. Biomed. Mater. Res. A*, 2014, **102**, 1092–101.
89. A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, *Cell*, 2006, **126**, 677–689.
90. Y.-R. V Shih, K.-F. Tseng, H.-Y. Lai, C.-H. Lin, and O. K. Lee, *J. Bone Miner. Res.*, 2011, **26**, 730–8.
91. J. R. Tse and A. J. Engler, *PLoS One*, 2011, **6**, e15978.
92. J. P. Winer, P. A. Janmey, M. E. McCormick, and M. Funaki, *Tissue Eng. Part A*, 2009, **15**, 147–54.
93. J. S. Park, J. S. Chu, A. D. Tsou, R. Diop, Z. Tang, A. Wang, and S. Li, *Biomaterials*, 2011, **32**, 3921–30.
94. A. Buxboim, K. Rajagopal, A. E. Brown, and D. E. Discher, *J Phys Condens Matter*, 2010, **22**, 194116.
95. P.-Y. Wang, W.-B. Tsai, and N. H. Voelcker, *Acta Biomater.*, 2012, **8**, 519–30.
96. E. F. Irwin, R. Gupta, D. C. Dashti, and K. E. Healy, *Biomaterials*, 2011, **32**, 6912–6919.
97. M. Witkowska-Zimny, K. Walenko, A. E. Wałkiewicz, Z. Pojda, J. Przybylski, and M. Lewandowska-Szumieł, *Acta Biochim. Pol.*, 2012, **59**, 261–4.
98. G. Ofek, V. P. Willard, E. J. Koay, J. C. Hu, P. Lin, and K. A. Athanasiou, *J. Biomech. Eng.*, 2009, **131**, 61011.
99. S. Musah, S. Morin A,, P. Wrighton J,, D. Zwick B,, S. Jin, and L. Kiessling L., *ACS Nano*, 2012, **6**, 10168–10177.
100. A. Raic, L. Rödling, H. Kalbacher, and C. Lee-Thedieck, *Biomaterials*, 2014, **35**, 929–40.

101.E. Csaszar, D. C. Kirouac, M. Yu, W. Wang, W. Qiao, M. P. Cooke, A. E. Boitano, C. Ito, and P. W. Zandstra, *Cell Stem Cell*, 2012, **10**, 218–29.
102.A. S. Curtis, J. V Forrester, C. McInnes, and F. Lawrie, *J. Cell Biol.*, 1983, **97**, 1500–6.
103.Z. L. Zhang, J. Tong, R. N. Lu, a M. Scutt, D. Goltzman, and D. S. Miao, *Bone Marrow Transplant.*, 2009, **43**, 69–81.
104.S. Jin, H. Yao, J. L. Weber, Z. K. Melkoumian, and K. Ye, *PLoS One*, 2012, **7**, e50880.
105.M. Jang, S. T. Lee, J. W. Kim, J. H. Yang, J. K. Yoon, J.-C. Park, H.-M. Ryoo, A. J. van der Vlies, J. Y. Ahn, J. A. Hubbell, Y. S. Song, G. Lee, and J. M. Lim, *Biomaterials*, 2013, **34**, 3571–80.
106.S. Vuoristo, S. Toivonen, J. Weltner, M. Mikkola, J. Ustinov, R. Trokovic, J. Palgi, R. Lund, T. Tuuri, and T. Otonkoski, *PLoS One*, 2013, **8**, e76205.
107.M. Kang and Y.-M. Han, *PLoS One*, 2014, **9**, e94888.
108.N. Jaiswal, S. E. Haynesworth, A. Caplan, and S. P. Bruder, *J Cell Biochem*, 1997, **64**, 295–312.
109.H. Bass Levy, Moises., Stern, Richard,, *Handbook of elastic properties of solids, liquids and gases*, Academic, San Diego, Calif.; London, 2001.
110.C. Yang, M. Tibbitt, L. Basta, and K. Anseth, *Nat. Mater.*, 2014, **13**, 645–652.
111.C. Chai and K. W. Leong, *Mol. Ther.*, 2007, **15**, 467–80.
112.J. Sambrook, E. Fritsch, and T. Maniatis, *Molecular Cloning*, 1982.
113.J. Blasiak, E. Gloc, K. Wozniak, and A. Czechowska, *Chem. Biol. Interact.*, 2004, **149**, 137–49.
114.R. D. Lentz, F. F. Andrawes, F. W. Barvenik, and A. C. Koehn, *J. Environ. Qual.*, **37**, 2293–8.
115.R. N. Palchesko, L. Zhang, Y. Sun, and A. W. Feinberg, *PLoS One*, 2012, **7**, e51499.
116.H. Abe, K. Hayashi, and M. Sato, *Data book on mechanical properties of living cells, tissues, and organs*, Springer Tokyo, 1996.

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Dr. Aneta M. Schaap-Oziemlakhas recently taken a postdoctoral position at Nencki Institute of Experimental Biology in Warsaw in Poland. She graduated at Erasmus MC University in Rotterdam in the group of Prof. Elaine Dzierzak. Her PhD studies were focused on development of hematopoietic stem cells (HSCs), applying both *in vitro* as well as *in vivo* models. Further, as the postdoctoral researcher she investigated proliferation and differentiation potential of mesenchymal stem cells (MSCs). Recently, her stem cell experience broadened to cancer stem cell biology, where she was able to successfully generate a mouse model with a human leukemia. Her growing interest in applying scaffolds to improve the leukemic xenograft mouse model, was further enhanced by investigations of MSCs growth and multilineage potential on synthetic biomaterials in the group of Dr. Patrick van Rijn at the department of BioMedical Engineering at the University Medical Center Groningen.

Philipp T. Kühn

After finishing his MSc in chemistry in Aachen, where Philipp Kühn focused on surface chemistry, he started his PhD in the group of Dr. Patrick van Rijn at the department of Biomedical Engineering in Groningen in 2013. There his work is focusing on the complex interactions between surfaces and biological systems. His current work is focused on the proliferation and differentiation behavior of MSCs at biointerfaces using complex gradient surfaces.

Dr. Theo G. van Kooten

Being educated in biomedical sciences, Dr. van Kooten started working at the interface between biomaterials, that are implanted in the human body, and cells and tissues interacting with these materials. Education has become an integral part of the work, with classes taught on biological evaluation and interface biology at the bachelor and master level within a Biomedical Technology curriculum. Current research is focusing on the development of approaches to control lens epithelial cell (trans)differentiation as a function of interaction with biomaterials, including self-assembling nanofiber systems. Also the foreign body reaction on the presence of degradable polymer systems is studied mainly through *in vitro* model systems involving macrophages and fibroblasts. Furthermore, macrophage activation in and by biofilms in relation to the biomaterial surfaces is being investigated.

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Dr. Patrick van Rijn is currently assistant professor and head of the research group Bio-inspired Synthetic Materials Science within the department of BioMedical Engineering at the University Medical Center Groningen. He is associated to the W.J. Kolff Institute for Biomedical Engineering and Materials Science in which he co-coordinates the research-line *"BioNanotechnology and Advanced Therapeutic Materials"*. His group focusses on the development of new biointerfaces, artificial virus structures and polymeric microgels for medical applications. Previously, he was an Alexander von Humboldt-fellow for Post-doctoral research in the group of Alexander Böker at the DWI-Leibniz-Institute for Interactive Materials, Aachen, Germany. He joined this institute in 2010 directly after receiving his PhD in the field of Supramolecular Chemistry with Jan H. Van Esch at Delft University of Technology.