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Interactions of cells with silk surfaces

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Polymers are often employed in tissue engineering to replace damaged extracellular matrix (ECM). During the last few decades silk proteins have been extensively investigated concerning their use as biopolymers for the generation of biocompatible, artificial scaffolds. Including the low or absence of immune-response and lack of cell toxicity, silk proteins present interesting properties useful for tissue engineering and organ repair. Since cell–matrix interactions define the behaviour of cells and posterior graft integration, this review is focused on the influence of surface properties of silk scaffolds (wettability, charge, elasticity and biodegradability) on the biological activity (adhesion, proliferation and/or migration) of cells cultured thereon. Further, it is highlighted how the origin of silk proteins (natural source, regenerated or recombinantly produced), as well as the scaffold morphology and its treatment/post-treatment influence the scaffold surface properties in the context of biomedical applications.

1. Introduction

Scientists are permanently searching for materials to support tissue repair processes. Such biomaterials should temporarily restore several properties of the natural extracellular matrix (ECM), until being absorbed and replaced by *de novo* ECM proteins.

Although the use of natural ECM components (*i.e.* collagens, fibronectins, elastin, *etc.*) has been broadly investigated, the

generation of scaffolds made of natural proteins presents a major drawback: the risk associated with contaminating viruses, bacteria, or prions among others. Therefore, during the last few decades, researchers have been studying new polymers for the generation of matrices for tissue engineering.^{1–3}

Scaffolds used in tissue repair should have several properties, such as support of cell attachment, the lack of toxicity, the absence of immune-response, mechanical properties similar to the engrafted tissues, and biodegradability. Since scaffolds made of silk proteins accomplish most of these desirable characteristics and since they have not been linked to viral, bacterial or prion

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logical production and processing of structural proteins as well as their application in biomedical and technical materials.

contaminants, silk proteins are envisioned for applications in wound healing and tissue repair.⁴

Graft integration is strictly dependent on good cell–material interactions. Therefore, we summarize the most important surface properties of silk scaffolds (presence of cell adhesion motifs, wettability, charge, elasticity) and report their influence on cell adhesion, proliferation and migration, as well as their biodegradation and a body's immune-response.

2. Silk sources for biomedical applications

Silk proteins are produced by most arthropods. Although the best-known examples are the silks of silkworms (*i.e. Bombyx mori*) and spiders (*i.e.* female orb-weaving spiders, such as *Araneus diadematus* or *Nephila clavipes* among others), there are reports on silks of other arthropods, such as ants (*i.e. Oecophylla smaragdina*),⁵ lacewings (*i.e. Mallada signata*),⁶ Caddisflies,⁷ or honeybees.⁸

Historically, Greeks and Romans used natural spider webs for covering wounds, and during the last few centuries degummed *Bombyx mori* fibroin was extensively utilized as a suture material.⁹ Nowadays, potential applications of natural silk fibres are under extensive investigation in a wide spectrum of biomedical applications, including surgical materials (*i.e.* sutures),¹⁰ or the use of simple fibrous silk scaffolds in tissue engineering.^{11,12}

Silk can be used “as-is”, taken directly from the animals. Further, silk proteins can be chemically “regenerated” (*i.e.* isolated from denatured/dissolved natural silk fibers), allowing preparation of scaffolds with non-fibrous morphologies such as films,^{13,14} non-woven meshes,^{15,16} sponges^{17,18} and hydrogels,^{19,20} expanding the potential applications of silks in tissue engineering by allowing the generation of complex matrices for 2D and 3D cell culture.

Alternatively, recombinant silk proteins can be employed,^{21–24} which is advantageous, since the silk primary structure can be simply modified to improve biocompatibility (*i.e.* by functionalizing silk proteins with RGD domains),²⁵ or to introduce new properties, *i.e.* by the generation of hybrids with ECM proteins, such as elastin,^{26,27} tropoelastin,²⁸ collagen and fibroelastin-like proteins²⁹ among others. In Table 1 an overview over employed natural, regenerated and recombinant silk proteins is given.

3. Silk in tissue engineering approaches

Adhesion plays a major role for a cell's metabolic activation, as well as for diminishing potential risks associated with unwanted biological responses (*i.e.* apoptosis or activation of oncogenes).³⁰

Cell adhesion is a complex phenomenon, which includes cell–cell (cell–cell junctions, occluding junctions, channel-forming junctions, and signal-relaying junctions), and cell–matrix interactions (cell–matrix junctions)³¹ all of which should be supported by a biomaterial used in tissue engineering.

Cell–matrix anchorage is mediated by transmembrane proteins (α and β integrins), which interact with specific amino acidic sequences (adhesion domains) present in most ECM proteins (*i.e.* fibronectin, collagen, elastin, *etc.*), yielding so-called *focal adhesions*.³² Since β -integrin subunits link proteins of the ECM with actin filaments from the cytoskeleton, *focal adhesions*

allow the direct intracellular sensing of distinct microenvironmental properties, such as the elasticity of the matrix.^{32–34}

In the case of cell–biomaterial interactions, three issues are important: (i) presence of specific motifs for cell–material anchorage, where specific domains for cell attachment present on the biomaterial surface are recognized by cellular integrins generating *focal adhesions* between a cell and the material surface (Fig. 1);³⁵ (ii) unspecific interactions between cells and the material, through ECM proteins adsorbed to the scaffold surface mediated by its physicochemical properties (*i.e.* charge, wettability) (Fig. 2);³⁶ (iii) morphological interactions between cells and the surface, where cell anchorage is mediated by the biomaterial's topography (Fig. 2).^{37,38}

4. Cell attachment to silk surfaces

Weak cell attachment to silk scaffolds has been reported in the past, such as for the osteoblast-like cell line Saos-2 which showed 50% less attachment to films made of *B. mori* silk fibroin than to treated cell culture plates,³⁹ or as for BALB/3T3 fibroblast attachment to films and hydrogels made of the recombinant spider silk protein eADF4(C16) in comparison to cell adhesion observed on treated cell culture plates (approximately 60% and 90% less adhesion, respectively).³⁷ Detachment forces of human umbilical vein endothelial cells (HUVEC) cultured on *B. mori* silk fibroin films are approximately 35% lower than on cell culture plates.⁴⁰ Moreover, cDNA microarray studies showed that MG63 cells (osteoblastogenic lineage) up-regulated the production of ECM proteins in relation to cell adhesion (collagen type-I production and fibronectin), when they were cultured in the presence of silk fibroin surfaces (*B. mori*).⁴¹ Similar results have been obtained by our group culturing BALB/3T3 fibroblasts on films made of eADF4(C16).³⁷ There, collagen type-I is up-regulated by approximately 80% compared to type-I collagen production of cells cultured on treated cell culture plates, suggesting that collagen and/or fibronectins are likely required to mediate the cell–surface anchorage in order to allow the survival of cells cultured on silk films.

Cells cultured on silk films and hydrogels typically maintain their spherical shape and generate micro-aggregates, since cell–cell anchorage is stronger than cell–matrix interactions.³⁷ These cell-spheroids are very weakly bound to silk surfaces, and, therefore, can be easily released upon low mechanical forces.³⁷ The weak cell attachment to silk scaffolds is based on the lack of specific domains for cell adhesion in most silk proteins (such as the typical adhesion motif GEFYFDLRLKGDK found in Collagen IV, YIGSR in laminin, and PHSRN and RGD in fibronectin, as well as specific glycosylation patterns).⁴² However, natural silk fibroin from *Antheraea mylitta* contains the amino-acid sequence RGD. Scaffolds made of this silk protein show much better cell adhesion than other silk scaffolds, being similar in adhesion and proliferation to films made of fibronectin, mostly attributed to the presence of this RGD-sequence in the silk primary structure.⁴³

In order to improve silk scaffolds concerning cell attachment, blends of silk and ECM proteins or the functionalization of silk proteins with the tri-peptide RGD or other adhesion domains have been employed.^{25,39,44–46}

Table 1 Most commonly used silk types in *in vitro* cell culture and *in vivo* tissue repair

Silk type and source	Recombinant protein	Repetitive sequence	Relevant characteristics for tissue repair	Reference
Fibroin, <i>Bombyx mori</i>	No	GAGAGS	Tested <i>in vitro</i> and <i>in vivo</i> . No toxicity detected. Low/no immune-response	1
Fibroin, <i>Antheraea mylitta</i>	No	GA sequences and Poly A blocks	Contains RGD sequences.	43
Spidroin, <i>Nephila clavipes</i> and <i>Nephila</i> spp.	No	GA sequences and Poly A blocks	Tested <i>in vitro</i> and <i>in vivo</i> . No toxicity detected.	52
eADF4(C16) (from <i>Araneus diadematus</i>)	Yes	GGX sequences and Poly A blocks	Tested <i>in vitro</i> . No toxicity detected.	37
4RepCT (from <i>Euprostheno australis</i>)	Yes	Poly A blocks	Tested <i>in vitro</i> . No toxicity detected. Good cell adhesion and proliferation.	21
AmelF3 (from honeybee)	Yes	GA sequences and Poly A blocks	Tested <i>in vitro</i> . No toxicity detected.	8
Silk-elastin like protein (SELP-47K)	Yes	GAGAGS sequences from silk fibroin and GVGVP from elastin	Tested <i>in vitro</i> . No toxicity detected.	27
Genetically engineered spider silk block copolymers (from <i>Nephila clavipes</i>)	Yes	GAGAAAAGGAG and GGX blocks	Tested <i>in vitro</i> . No toxicity detected.	88
Genetically engineered spider silk block copolymers (from <i>Nephila clavipes</i>) modified with RGD domains	Yes	GAGAAAAGGAG and GGX blocks	Contain RGD sequences.	25
Genetically engineered silkworm silk functionalized with fibronectin domains (from <i>Anaphe</i> spp.)	Yes	Block sequences [(AAG) ₆ ASTGRGDSPAAS] _n and [(AG) ₉ ASTGRGDSPAAS] _n	Tested <i>in vitro</i> . No toxicity detected. Improved cell adhesion in comparison to wild type.	89
rS1/9 from <i>Nephila clavipes</i>	Yes	GGX sequences and Poly A blocks	Tested <i>in vitro</i> . No toxicity detected.	90

It is important to note that the presence of RGD domains (genetically or chemically coupled) incorporated in silk proteins is a basis but is not sufficient to stimulate cell attachment or to generate *focal adhesions*. Further, the spacing of such binding-domains on a scaffold's surface is of high importance, with an optimal distance between individual adhesion domains being below 70 nm.⁴⁷

4.1. Charge

In the absence of specific cell adhesion domains, positive surface charges play an important role in cell attachment. This issue has been addressed by attaching positively charged poly-L-lysine to the negatively charged cellular glycocalyx,⁴⁸ improving cell adhesion to films made of hyaluronic acid.⁴⁹

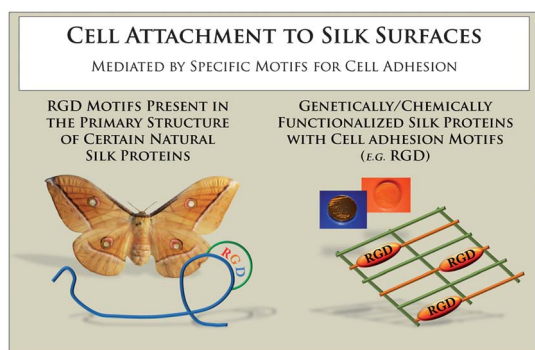


Fig. 1 Cell anchorage to silk matrices mediated by specific interactions.

The density of the charges is highly important for cell attachment, with a moderate density of positive charges improving cell adhesion, supported by the slight increase of hydrophilicity.^{50,51} High densities of charges render the surface very hydrophilic, which then diminishes cell adhesion.⁵¹

Although a direct correlation between surface charge and cell attachment has not been studied on silk matrices, it is interesting to note that low cell adhesion has been observed in the case of *B. mori* fibroin, which presents a high density of negative charges (pI of 4.39), as well as in the case of the engineered spider silk protein eADF4(C16) (pI of 3.48) (Table 2). Negative surface charges interfere with cell–matrix interactions, hindering cell spreading and posterior production of ECM proteins.

Silk scaffolds made of the engineered spider silk protein 4RepCT 21 (pI of 9.30) or native spider dragline silk from *Nephila* spp.⁵² comprising proteins with pIs of 10.22 and 6.47 (for spidroin 1 and 2, respectively) (Table 2) show good cell attachment and proliferation, probably due to cell–matrix interactions mediated by increased type-I collagen (pI 5.46) and fibronectin (pI 5.60) adsorption.

4.2. Wettability

Cell adhesion depends on the wettability of a material's surface. The degree of wetting is determined by a balance between adhesive and cohesive forces, determined by the hydrophobicity/hydrophilicity of the surface.⁵⁰

Studies have shown that cell adhesion is high on weakly hydrophilic surfaces (with water contact angles of approximately

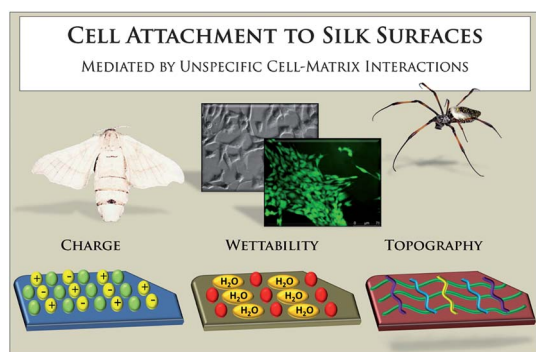


Fig. 2 Cell anchorage to silk matrices mediated by unspecific interactions.

60°), due to an increased adsorption of ECM proteins.^{50,53,54} Otherwise, extremely hydrophilic or extremely hydrophobic surfaces are traditionally used to avoid cell adhesion.^{50,53,54} Examples of surfaces with low/no cell adhesion are non-treated polystyrene cell culture plates (water contact angle of approximately 90°) or commercially available highly hydrophilic hydrogels, where cell adhesion is less than 10% of that of traditional treated cell culture plates (*i.e.* Corning® ultra low attachment surfaces).

Therefore, tuning the surface wettability can modulate cell adhesion. This probability has been shown by using thermoresponsive materials (*i.e.* PIPAAm polymers) which switch their wettability with temperature, incrementing triggered hydrophobicity as the mechanism to induce cell detachment.⁵⁵

In the case of silk films, traditionally reported values of water contact angles (*i.e.* films made of eADF4(C16) and/or *B. mori*) can be found between 50° and 70° depending on the film's post-treatment.^{37,39,56} In the case of films made of *B. mori* silk fibroin, wettability can vary with the processing temperature, where films cast at 50 °C present lower contact angles (higher wettability) than those cast at 20 °C or 70 °C.⁵⁶ One example of tuning the wettability of silk surfaces was the use of mica or sacrificial colloidal crystal substrates to cast *B. mori* silk films on, obtaining films with different contact angles (less than 40° or more than 100°, respectively).⁵⁷ Moreover, according to Sofia *et al.* *B. mori* films cast from aqueous solutions exhibited water contact angles of 55° ± 2°, while those cast from hexafluoroisopropanol (HFIP) showed water contact angles of 67° ± 2°.³⁹

The wettability of silk surfaces can be further modulated by functionalizing the scaffold's surface with polar/non-polar groups,⁵³ *i.e.* by plasma treatment of silk scaffolds with either O₂ or CH₄.⁵⁸ After plasma treatment, a significant increase of adhesion and spreading of keratinocytes was observed with respect to the control (without plasma treatment). Interestingly, cell adhesion to scaffolds treated with O₂-plasma was notably better than to CH₄-plasma-treated ones. However, the influence of surface wettability on cell adhesion seems to be a cell-dependent phenomenon, since growth of human fibroblasts cultured on untreated, CH₄-plasma-treated or O₂-plasma-treated matrices was nearly indistinguishable.⁵⁸

4.3. Topology

A scaffold's surface topography has a strong influence on cellular morphology, polarity and cytoskeleton reorganization.⁵⁹

Since cells typically adhere weakly to silk surfaces, their topography could play a role in cell adhesion.³⁷ When pig iliac endothelial cells were cultured on films or nano-fibrous scaffolds made of *B. mori* fibroin, cells adhered approximately 40% better to fibrous matrices.⁶⁰ According to our results, protrusion of cellular phylopodia/lamellipodia in electrospun fibres could be responsible for the improvement of cell adhesion and posterior proliferation on silk fibre meshes compared to that on flat surfaces (films).³⁷ Furthermore, cells cultured on electrospun meshes with different fibre diameters (between 150 and 680 nm) showed increased proliferation rates (decreasing of doubling time) with increasing fibre diameters. This finding is likely based on the fact that the organization of the cytoskeleton and the spacing between the electrospun fibres is strictly related.³⁷

The surface roughness of silk scaffolds has no clear effect on adhesion and/or posterior proliferation. When cells were cultured on RGD functionalized silk films (*B. mori*) with different roughness, cell orientation and alignment were influenced, but not cell adhesion or proliferation.^{14,61} Similarly, primary human dermal fibroblast cultured on films or electrospun meshes made of the positively charged 4RepCT proteins showed only minor differences in terms of adhesion and proliferation.²¹ In both cases, surface charge had a much higher impact on interactions with cells than the scaffold topography.

5. Surface properties of silk scaffolds in context with immune-reactivity

An intriguing property of silks is the low level of immune-response upon scaffold engraftment.^{4,62} Although fibroins and/or spidroins are typically acknowledged as non-immune reactive, one publication indicates the presence of antibodies against the terminal domains of spider silk proteins.⁶³ Most of the *in vivo* research performed with silk engraftments shows a weak inflammatory response.⁶⁴ However, mostly inflammatory responses have been connected to sericin proteins, which reflect

Table 2 pI and number of charged amino acids (source: ExPASy.org)

Protein	Number of positively charged amino acids	Number of negatively charged amino acids	pI
Fibroin heavy chain (<i>Bombyx mori</i>)	26	55	4.39
Fibroin light chain (<i>Bombyx mori</i>)	15	22	5.06
Fibroin (<i>Antheraea mylitta</i>)	26	41	5.05
ADF4 (<i>Araneus diadematus</i>)	2	6	4.14
ADF3 (<i>Araneus diadematus</i>)	4	2	8.51
ADF2 (<i>Araneus diadematus</i>)	3	3	6.74
ADF1 (<i>Araneus diadematus</i>)	7	6	8.07
Spidroin 1 (<i>Nephila clavipes</i>)	20	4	10.22
Spidroin 2 (<i>Nephila clavipes</i>)	4	4	6.47
Recombinant spider silk eADF4(C16)	1	16	3.48
Recombinant spider silk 4RepCT	5	2	9.30
Fibronectin (human)	203	253	5.46
Collagen type I alpha 1 (human)	128	141	5.60

the glue between fibroin-fibres of a silk cocoon, and therefore, their use in silk scaffolds has to be avoided.⁶⁵ The reported immune activity after engraftment of scaffolds made of regenerated silk fibroin^{64,66} could also be related to scaffold toxicity⁶⁷ based on solvent remnants in the material's matrix from the treatment and/or post-treatment of the silk scaffolds.^{64,66} The remaining solvent induced a higher expression of TNF- α , INF- γ , IL-6, IL-4, and IL-13 after an *in vivo* implantation of fibroin particles made of *B. mori* fibroin prepared in HFIP compared to those prepared in an aqueous solution.⁶⁴

One likely explanation for the high immune-compatibility of silk scaffolds is the low macrophage adhesion and spreading on silk films made of *B. mori* fibroin and the adsorption of certain proteins involved in the activation of the immune-response (*i.e.* C3 complement, among others).⁶⁸

6. Toxicity

Besides the weak immune-response, a remarkable characteristic of silks is the complete absence of or at highest only low toxicity. Although differences in terms of scaffold integration (*i.e.* vascularization, degradation grafts) have been attributed to the contact with organic solvents during post-treatment,⁶⁶ most literature reports the absence of toxicity of silk proteins or silk scaffolds (Table 1). In this context it should be mentioned that the potential for cross-seeding of amyloidogenic peptides with silk structures might exist, since amyloid peptides have been shown to be cross-seeded from *B. mori* silk fibroin followed by moderate amyloid accumulation in tissues *in vivo*.⁶⁹

7. Mechanical properties of silk scaffolds used in biomedicine

Mediated by active *focal adhesions*, surface elasticity is sensed by attached cells in a process known as mechanotransduction.⁷⁰

As commented previously, integrins are constituted by two associated glycoproteins (α and β subunits), which exhibit an intracellular and an extracellular domain. In case of activated *focal adhesions*, the intracellular part of the β -subunit binds to actin microfilaments (*i.e.* via the G-protein talin), allowing the transduction of the external mechanical stimuli into intracellular responses.^{32,71} Cells dynamically and constantly sense and tune the matrix surface elasticity, regulating their activity, as well as the properties of the surrounding ECM, such as stiffness and pore size distribution, by modifying the synthesis of *de novo* ECM proteins, regulating ECM protein degradation, and/or initiating their crosslinking.^{32,70,71}

Clearly, cells can recognize mechanical properties of a material's surface, which influences their metabolic activity, proliferation, migration, or differentiation.^{32,34,70} Regarding the mechanical properties of biomaterials used in tissue engineering it is therefore necessary to generate matrices with similar mechanical properties as found in tissues or organs. Gilbert *et al.*⁷² showed that stem cells pre-cultured on a 2D hydrogel surface with similar rigidity as the engrafted tissue (stiffness of around 10 kPa, measured as Young's modulus), presented a much better *in vivo* engrafting than those cultured on treated cell culture plates (stiffness of approximately 3 GPa), which exhibited no posterior *in vivo* expansion and differentiation.

Mismatched mechanical properties can potentially activate unwanted biological responses, like those associated with a pathology or disease, *i.e.* in the case of a healthy liver which exhibits a stiffness between 1 and 5 kPa, a fibrotic response (*i.e.* triggered by hepatitis) can be activated, incrementing values of stiffness up to approximately 70 kPa (fibrotic liver).^{73,74}

In natural silk fibres, surface stiffness is in the regime of GPa.⁷⁵ Since most tissues and organs from vertebrates exhibit values of stiffness less than 200 kPa,⁴ the use of natural silk fibres as scaffolds for tissue engineering is inadequate from a mechanical point of view.

In order to tune the mechanical properties of silk scaffolds, blends with elastic proteins of the extracellular matrix are an appropriate tool. Films made of blends of *B. mori* fibroin and tropoelastin showed an increased biocompatibility and cell adhesion in comparison to plain *B. mori* silk films.²⁸ However, only films made of 90% tropoelastin and 10% silk fibroin showed clearly different mechanical properties in contrast to films made of 100% silk.²⁸ In the case of blends of elastin and silk fibroin (*B. mori*), a clear diminution of the stiffness was observed already when using a 50/50 blend (with the Young's modulus changing from approximately 25 MPa to 5 MPa).⁷⁶

For replacing soft tissue the best option might be silk hydrogels.^{20,77} Gels made of the recombinant spider silk eADF4(C16) exhibited Young's moduli between 1 kPa and 20 kPa,⁷⁷ mimicking mechanical properties of very soft tissues, such as liver tissue (1.5–5.0 kPa)⁷⁸ or the nucleus pulposus in intervertebral discs (5.8 kPa).⁷⁹

However, when human mesenchymal stem cells were entrapped in silk hydrogels (*i.e.* made of *B. mori* fibroin) they exhibited only a short phase of proliferation followed by a decay stage,²⁰ which could be due to solid stress⁸⁰ and/or pore coarsening at the cell–hydrogel interface as a consequence of cell proliferation.^{73,81}

8. Biodegradation of silk scaffolds

With the exception of permanent prosthesis, biodegradation of artificial matrices used in tissue engineering is a requisite. Scaffold degradation allows the replacement of the biomaterial residues by *de novo* synthesized extracellular matrix proteins and the morphogenesis of new tissue required for proper wound healing.⁸²

Biodegradation of artificial scaffolds is mediated by cell–matrix interactions (*i.e.* cell-secreted and/or membrane-associated proteases).^{83,84} Most of the proteases in tissue repair are endopeptidases from the family of metalloproteinases (MMPs) which are characterized by the involvement of Zn²⁺ or Ca²⁺ ions in their active site. MMPs are naturally responsible for degradation, synthesis and activation of ECM proteins.^{83–85}

Concerning degradation of silk scaffolds by MMPs, not much is known. Studies on the biodegradation of silk scaffolds (*i.e.* made of *B. mori* silk fibroin) often used “model proteases” such as protease XIV (from *Streptomyces griseus*), Collagenase IA (from *Clostridium histolyticum*), protease mycolysin/pronase (from *Mycolysin streptomyces*), trypsin, and α -chymotrypsin, which however, do not represent the proteases of the *in vivo* micromilieu during wound healing.

With respect to *in vivo* biodegradation of silk scaffolds (made of *B. mori* fibroin), experiments show the partial degradation of

fibroin particles (intramuscularly implanted) in a timeframe of 6 months to one year, indicating that silk fibroins (when processed into a solid structure) are very slowly degraded *in vivo*.⁶⁴ The high crystallinity of the silk protein structure in scaffolds could be one reason for restricting the interactions between MMP active sites and the protein backbone. Since cell attachment to silk scaffolds is typically low, and since the activity and secretion of MMPs is mediated by the presence of active *focal adhesions*,⁸⁶ this represents another reason for the slow biodegradation of silk matrices.

9. Outlook: replacing the extracellular matrix by silk materials

The extracellular matrix (ECM) is a complex network of proteins and polysaccharides surrounding cells in tissues. The ECM defines the extracellular micro-architecture in terms of adhesion, maintains the tensile strength, facilitates cell migration, guides tissue morphogenesis and repair, regulates activity of secreted proteins, and is involved in cell–cell communication acting as a quasi co-receptor.

In tissue engineering approaches, scaffolds made of biocompatible polymers should replace the function of the ECM.^{35,87} In order to mimic distinct properties of the natural ECM, scaffolds made of silk proteins (natural, regenerated and/or recombinantly produced) have been extensively studied. Surface properties of silk scaffolds are likely responsible for their low immune-reactivity. However, the absence of specific domains for cell attachment, as well as the presence of negatively charged amino acid residues in some silk proteins could be counterproductive for the design of novel scaffolds useful for biomedical applications.

In order to avoid complications such as contaminants, chemically and genetically modified silks gain more and more impact in biomaterials research. In the context of good mechanical properties and slow biodegradation, the genetic modification of silk proteins seems to be an excellent way to achieve functional similarities to compounds of the natural ECM.

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