FORUM CRITICAL REVIEW

# Self-Assembled Nanomaterials for Chronic Skin Wound Healing

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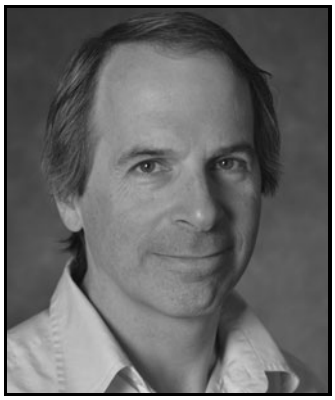

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**Significance:** Chronic wounds are one of the major burdens of the U.S. health care system with an annual cost of $31.7 billion and affecting an estimated 2.4–4.5 million people. Several underlying molecular and cellular pathophysiological mechanisms, including poor vascularization, excessive extracellular matrix (ECM) degradation by proteases, decreased growth factor activity, and bacterial infection can lead to chronic wounds. More effective wound therapies need to address one or more of these mechanisms to significantly advance wound care.

**Recent Advances:** Self-assembled nanomaterials may provide new therapeutic options for chronic wound healing applications as those materials generally exhibit excellent biocompatibility and can bear multiple functionalities, such as ECM-mimicking properties, drug delivery capabilities, and tunable mechanics. Furthermore, self-assembled nanomaterials can be produced at low cost, and owing to their ability to self-organize, generate complex multifunctional structures that can be tailored to the varying sizes and shapes of chronic wounds. Self-assembled nanomaterials have been engineered to serve as wound dressings, growth factor delivery systems, and antimicrobials.

**Critical Issues:** As there are many different types of self-assembled nanomaterials, which in turn have different mechanisms of self-assembly and physiochemical properties, one type of self-assembled nanomaterials may not be sufficient to address all underlying mechanisms of chronic wounds. However, self-assembled nanomaterials can be easily tailored, and developing multifunctional self-assembled nanomaterials that can address various targets in chronic wounds will be needed.

**Future Directions:** Future studies should investigate combinations of various self-assembled nanomaterials to take full advantage of their multifunctional properties.

**Keywords:** nanomaterials, self-assembled peptides, drug delivery, scaffolds, antibiotics

## SCOPE AND SIGNIFICANCE

Chronic wounds often fail to progress through the normal wound healing process and thus do not heal in a timely and orderly manner for more than a month.[1] Some of the underlying molecular and cellular pathophysiological mechanisms include poor vascularization, excessive extracellular matrix (ECM) degradation by proteases, decreased growth factor activity, and bacterial infection. Several types of wound dressings and drug delivery systems have



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been developed to address these problems and have been discussed in other review articles.[1–3] Emerging technologies based on self-assembled nanomaterials, which are discussed here, provide new opportunities for chronic wound healing applications, owing to their great biocompatibility, ECM-mimicking properties, drug delivery capabilities, and easily tunable mechanics.

## TRANSLATIONAL RELEVANCE

Novel biomaterials are continuously being developed to enhance chronic wound healing; however, some limitations exist, such as their cost and complexity of manufacture. Self-assembled nanomaterials may overcome some of these limitations, as they are cost-effective to produce, and can create complex and multifunctional structures via self-assembly.[4] Furthermore, the multifunctional aspects of self-assembled nanomaterials make them amenable to targeting multiple pathways in chronic wounds. Self-assembled nanomaterials may also be incorporated into existing wound dressings or used in combination with other technologies used for treating chronic wounds.

## CLINICAL RELEVANCE

Chronic wounds severely burden the U.S. health care system, costing $31.7 billion/year and affecting an estimated 2.4–4.5 million people.[5,6] Open wounds are prone to infection, which can lead to life-threatening sepsis or amputation of the affected limb.[7] Advanced methods to help wound healing, such as bioengineered skin substitutes, negative pressure therapy, and hyperbaric oxygen therapy, often fail to achieve complete healing since wound size and location vary significantly among individuals, and these approaches only partially address the relevant biological mechanisms.[5,7] Self-assembled nanomaterials may address a critical need to develop better treatments targeting multiple mechanisms in chronic wounds.

## OVERVIEW

### Wound healing targets for self-assembled nanomaterials

Skin wound healing is an orderly multiphase process consisting of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling.[5] However, chronic wounds are often stalled at the inflammatory phase.[5,7] In chronic wounds, macrophages fail to switch from a proinflammatory M1 phenotype secreting high levels of inflammatory mediators to an anti-inflammatory or pro-resolution M2 phenotype.[7] The excessive inflammatory cytokine signaling increases the influx of neutrophils, which in turn release metalloproteinases and elastases. These abnormal conditions can impair wound healing through the following mechanisms, which can be potentially addressed by self-assembled nanomaterials (Fig. 1): (1) loss of endogenous ECM, (2) impaired growth factor activity, and (3) bacterial infection.

In the normal wound healing process, the temporary ECM that constituted of fibrin of the granulation tissue is progressively invaded by endothelial cells and fibroblasts as it is being replaced by collagen to reconstitute damaged or lost tissue. However, elevated metalloproteinases and elastases in chronic wounds result in the continuous degradation of the ECM scaffold thus preventing cellular migration. Since many self-assembled nanomaterials are made of the same building blocks as natural peptides and mimic ECM by incorporating specific amino acid sequences,[4] they can provide a matrix that substitutes for the damaged or lost tissue in wounds.

Metalloproteinases also degrade endogenous growth factors and bioactive peptides, thus further impeding cellular proliferation, differentiation, and migration, responses that are associated with wound healing.[5] The activity of growth factors and cytokines may also be impaired due to the unusually high level of reactive oxygen species.[8] Exogenous topical growth factors are also susceptible to the same proteolytic processes, which may explain their limited success as therapeutics, with the exception of platelet-derived growth factor (becaplermin).[9] As discussed further below, self-assembled nanomaterials have been used as drug delivery vehicles that can shelter the bioactive compounds from degradation.[10]

Chronic wounds are at high risk of bacterial infection and colonization, an increasing problem due to the emergence of antibiotic-resistant pathogens. Although systemic antibiotics are often used in cases of large wounds such as burns, topical application of antimicrobials has fewer systemic side effects and lower occurrence of antimicrobial resistance.[11] Self-assembled nanomaterial-based nanoparticles allow sustained and controlled release of antibiotics, thus minimizing the peak and trough variation in antibiotic levels that decreases effectiveness and increases the chance of antibiotic resistance.

### Types of self-assembled materials

Self-assembled nanomaterials, especially synthetic self-assembled nanomaterials, are designed

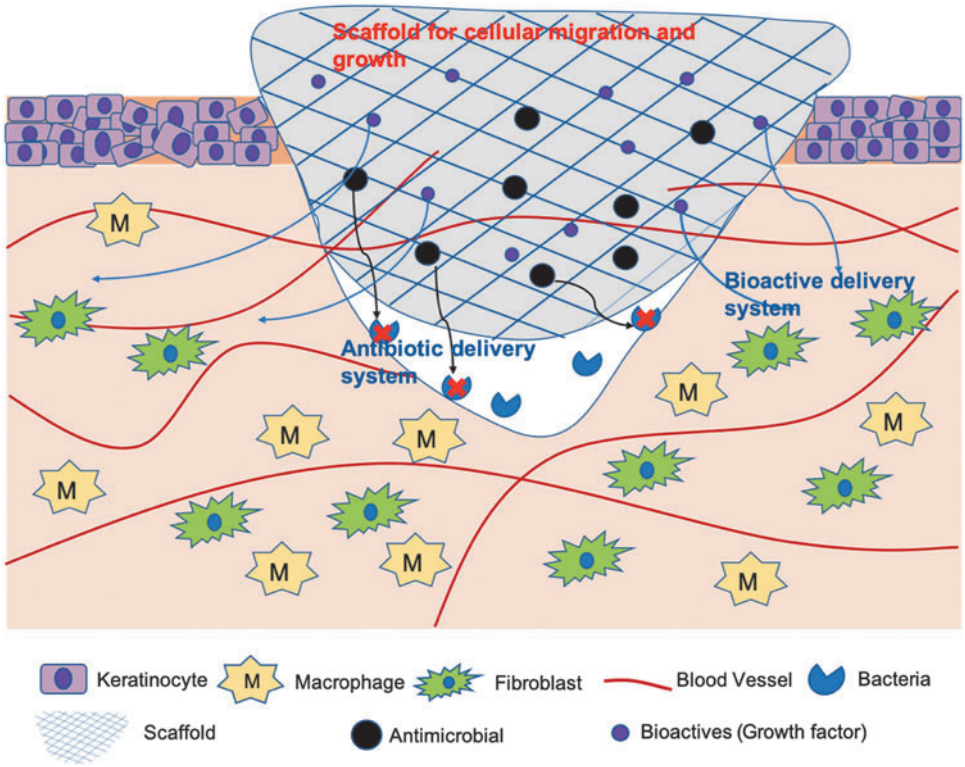


**Figure 1.** Schematic of self-assembled nanomaterials on skin wounds. Self-assembled nanomaterials can serve as (1) 3D scaffolds where cells can migrate and proliferate; (2) a delivery system where bioactive compounds such as growth factors can be encapsulated; (3) and an antibiotic delivery system to prevent bacterial colonization and infection. Self-assembled nanomaterials can be designed or combined to include multiple properties at the same time. 3D, three dimensional.

"from scratch" and thus are extremely versatile.[12,13] Figure 2 summarizes the main types of synthetic self-assembling structures, which generally consist of peptides, polymers, and metal-based structures.

*Self-assembled peptides.* Self-assembled peptides (SAPs) consist of short amino acid chains that form nanofibrous hydrogels, nanoparticles, and nanotubes.[14] Similar to the process of natural ECM formation, synthetic short peptide chains self-assemble via noncovalent interactions, such as hydrogen bonding, van der Waals forces, hydrophobic interactions, and $\pi$–$\pi$ stacking.[12] The fibrils can be further stabilized via crosslinking using physical or chemical methods.[15] The amino acid sequence governs the secondary structure of the peptide, usually an $\alpha$-helix or a $\beta$-sheet. The $\beta$-sheet motif consists of alternating hydrophobic and hydrophilic amino acids, which stabilize into a $\beta$-sheet via hydrophobic interactions between the intermolecular hydrophobic interfaces and ionic interactions between hydrophilic interfaces. It is the most common mechanism of nanofiber assembly that results in peptide-based hydrogels. These hydrogels exhibit good injectability and tunable mechanical properties.[15]

One of the simplest SAPs is diphenylalanine (FF), which self-assembles into hydrogels. Changing FF concentration can readily tune the hydrogel mechanics.[15] Another example of SAPs is EAK16-II (AEAEAKAKAEAEAKAK), which self-assembles to form hydrogen-bonded $\beta$-sheet nanofiber hydrogels.[16] Some of the most widely explored SAPs are RADA16-I (Ac-RADARADARADARADA-$NH_2$) and RADA16-II (Ac-RARADADARARADADA-$NH_2$),[17] which also form $\beta$-sheet structures in aqueous solutions. Recently, other types of SAPs, including crosslinked ultrashort peptides (LIVAGKC),[18] multidomain peptides consisting of 16-amino acids of $K_2(SL)_6K_2$,[19] and N-fluorenylmethyloxycarbonyl SAPs (Fmoc-SAPs),[20] have been used for wound healing.

*Peptide amphiphiles.* Peptide amphiphiles contain four distinct domains: (1) a bioactive domain that binds specific receptors, thus promoting cell adhesion or other cellular responses, (2) a polar domain that confers solubility in aqueous environments, (3) a stabilization domain, that is, often

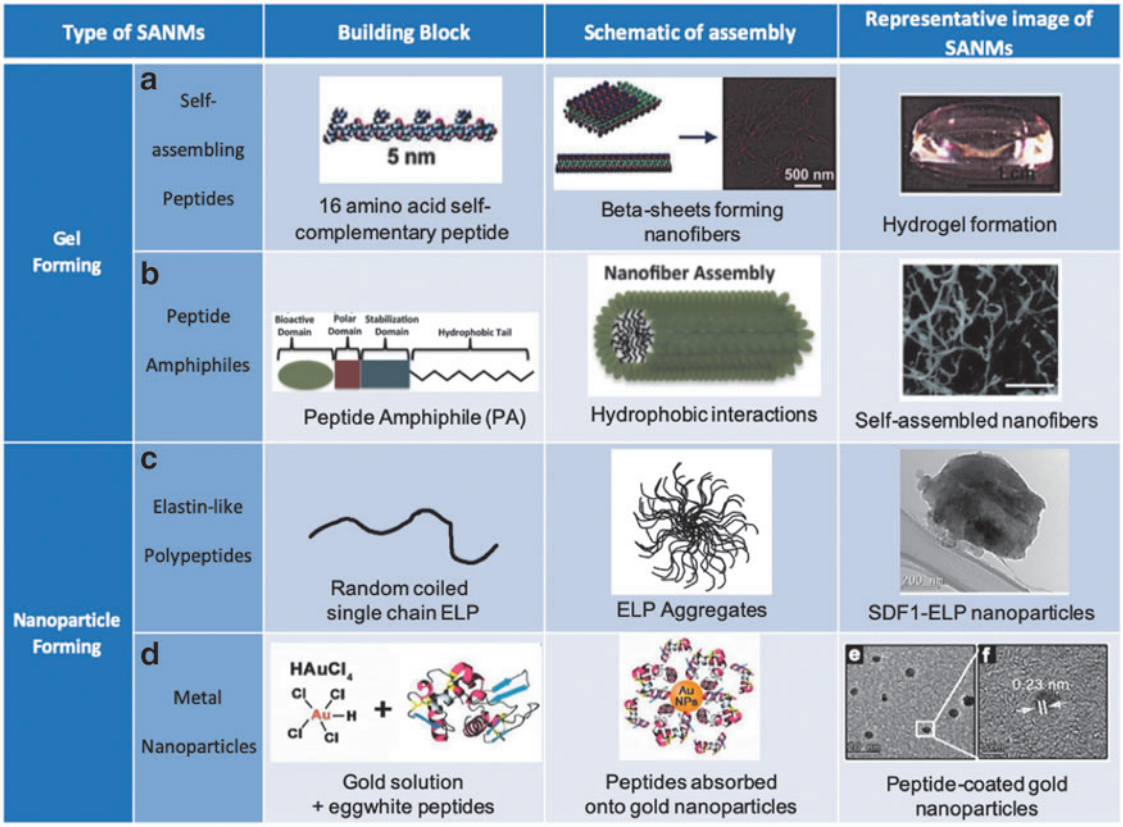


**Figure 2.** Major types of self-assembled nanomaterials used in wound healing studies. **(a)** The ionic self-complementary peptides form stable $\beta$-strand and $\beta$-sheet structures, which undergo self-assembly to form nanofibers. These nanofibers form interwoven matrices that further form a scaffold hydrogel with very high water content. Adapted from Ref.[13]. **(b)** Peptide-amphiphiles contain four distinct engineered regions. Hydrophobic tails help cylindrical micelle assembly. The stabilization domain is often a $\beta$-sheet forming sequence of amino acids. The polar domain aids in solubility. A bioactive domain can be included to aid in cell adhesion, degradation, or growth factor presentation. Adapted from Ref.[12]. **(c)** ELP exists as monomers below a transition temperature and undergoes nanoparticle formation above the transition temperature. Reprinted from Ref.[41] with permission from Elsevier. **(d)** CEW was used to prepare the gold nanoparticles as a reducing and stabilizing agent. The MMT self-assemble on the surface of nanoparticles resulting in Au@CEW/MMT as an effective antibacterial agent. Adapted from Ref.[43] CEW, chicken egg white; ELP, elastin-like polypeptide; MMT, 2-mercapto-methylimidazole.

a $\beta$-sheet forming sequence, and (4) a hydrophobic tail that enables micelle assembly.[12] Peptide amphiphiles self-assemble into nanofibers with a cylindrical geometry via intermolecular hydrogen bonding, which then forms a nanofibrous hydrogel that exhibits viscoelastic properties, topography, and bioactive signaling reminiscent of native ECM.[21] The resulting three-dimensional (3D) structure is easily tunable by changing the amino acid sequence.

*Elastin-like polypeptides.* Another class of SAPs is elastin-like polypeptides (ELPs), which are derivatives of tropoelastin with pentapeptide repeats of valine/proline/glycine/X/glycine, where X can be any amino acid except proline.[9,22] ELPs undergo a reversible thermal transition above a certain temperature, which is usually designed to be between 20°C and 70°C by adjusting the number of pentapeptide repeats, pH, ionic strength, and the chosen X amino acid.[23] Above the transition temperature, ELPs fold into a $\beta$-spiral conformation and self-assemble via intrachain and interchain hydrophobic interactions. ELPs can also be engineered into fusion proteins incorporating bioactive peptides. The reversible self-assembly feature makes it possible to perform a relatively simple purification procedure, and also protects the bioactive molecule from proteolytic degradation when the fusion protein is in its nanoparticle form at physiological temperature.

## Mechanisms of nanomaterial self-assembly

There are various mechanisms by which self-assembly can be achieved. Most widely used self-assembled nanomaterials that form peptide-based hydrogels self-assemble spontaneously in physiological conditions.[12] On the contrary, enzymes can also be used to aid the self-assembling process.[15] When stronger mechanical properties are required,

physical or chemical crosslinking can be used.[15] In this section, we briefly overview some of the mechanisms that govern self-assembly (Fig. 3).

*Spontaneous self-assembly.* Most self-assembled materials spontaneously form structures due to noncovalent interactions upon changing environmental conditions, including pH, metal ion concentration, salt concentration, and temperature.[12] For example, adding Fmoc at the N-terminus of FF molecules, which themselves self-assemble into hydrogels, confers an additional level of control on the self-assembly process. The secondary structure formed from the modified peptides (Fmoc-FF) is dependent upon the charge on the molecules. By using a different pH at time of self-assembly, one can alter the protonation state of the carboxyl group at the C-terminus.[24] In acidic to neutral pH, Fmoc-FF molecules self-assemble into a nanofibrous $\beta$-sheet structure via amide/amide hydrogen bonding, which further forms a hydrogel via hydrogen bonding and $\pi$–$\pi$ stacking. In contrast, when the carboxyl group is deprotonated at pH 8.5, the secondary structure of self-assembled Fmoc-FF is $\alpha$-helical due to electrostatic repulsion between Fmoc-FF molecules, which yields a viscous solution and not a gel.

Metal ions can also be used to trigger self-assembly. Abul-Haija *et al.* used two different tripeptides, glycine-histidine-lysine (GHK), which

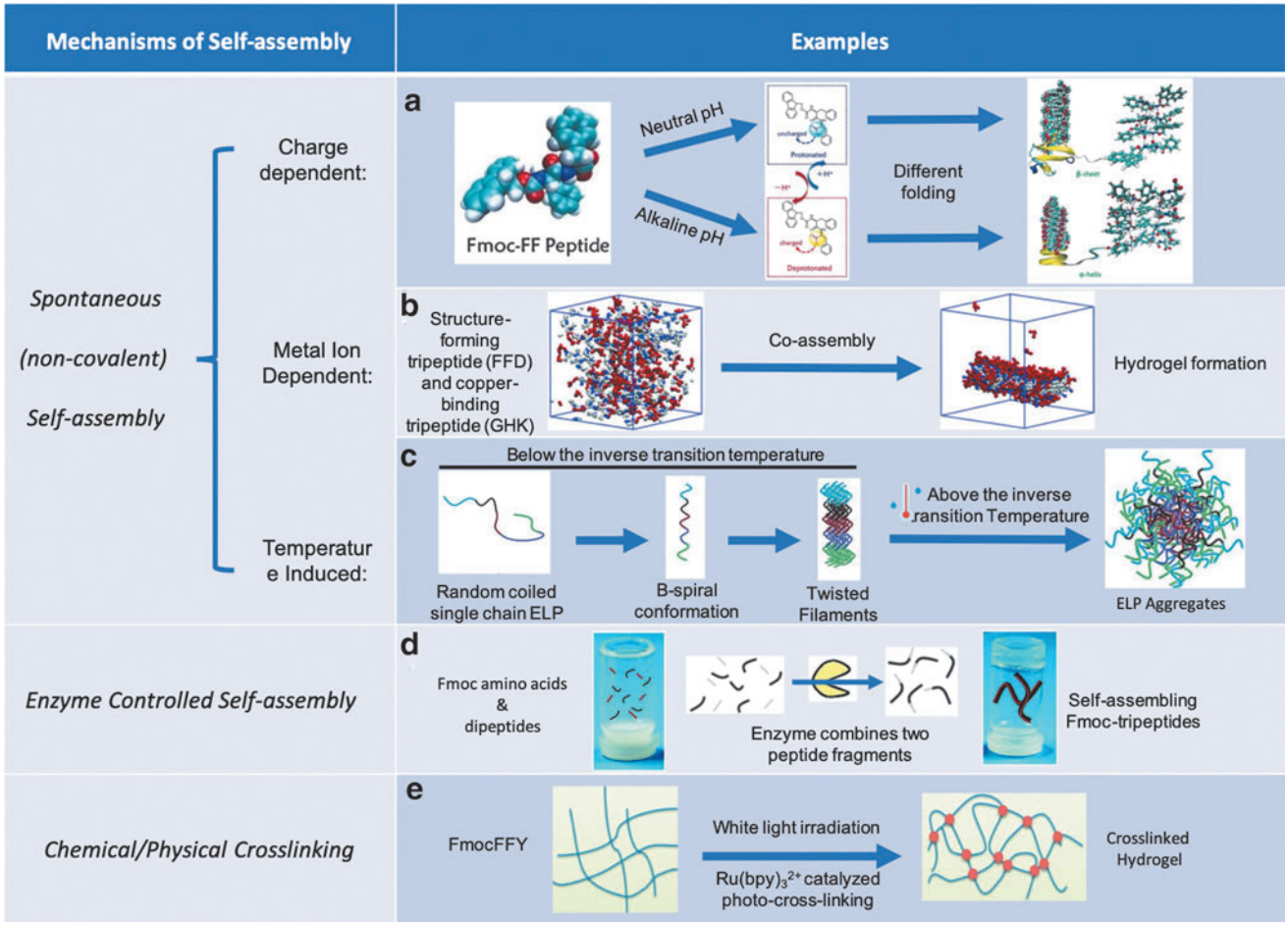


**Figure 3.** Representative mechanisms of self-assembly. **(a)** The Fmoc-FF monomers containing charged carboxyl groups can serve as a pH trigger for secondary structure transformation. The neutral Fmoc-FF dipeptide can form fibrous hydrogels, which consist of $\beta$-sheet structures. Adapted from Ref.[24]. **(b)** Computational time course for the self-assembly of coassembled nanostructures. When introducing GHK into an FFD system, a coassembly is observed where GHK peptides are organized on the surface of the FFD structure. Adapted from Ref.[25]. **(c)** Random coiled single ELP chains turn to a $\beta$-spiral conformation, which stack up against each other to form "twisted filaments" as they reach their transition temperature. Above the transition temperature, the twisted filaments associate with each other to form insoluble aggregates. Adapted from Ref.[10]. **(d)** Fmoc amino acids are enzymatically coupled to dipeptides to form Fmoc-tripeptides that self-assemble to higher order aggregates. Reprinted with permission from Ref.[26]. Copyright (2006) American Chemical Society. **(e)** Hydrogelation and $Ru(bpy)_3^{2+}$-mediated photocrosslinking enhance the mechanical stability of the FmocFFGGGY hydrogel. Reprinted with permission from Ref.[29]. Copyright (2013) American Chemical Society. FF, diphenylalanine; FFD, diphenylalanine-aspartic acid; GHK, glycine-histidine-lysine.

itself is not a gelator but a copper-binding peptide, and diphenylalanine-aspartic acid (FFD), which is a "structure-forming" peptide.[25] Although FFD peptides form hydrogels at pH 5, FFD and GHK do not self-assemble at neutral pH; however, when copper ions were added to the mixture, GHK and FFD spontaneously formed hydrogels at neutral pH.

Temperature can also affect self-assembly of self-assembled nanomaterials. The best example of temperature-sensitive materials are ELPs, which undergo spontaneous self-assembly above a certain "transition" temperature. Below that temperature, the ELPs exist largely as monomers, but when the temperature is increased above the transition temperature, ELPs fold into a $\beta$-spiral conformation and self-assemble via intrachain and interchain hydrophobic interactions to form nanoparticles.[23]

*Enzyme-catalyzed self-assembly.* Enzymes are biocompatible and offer mild reaction conditions (aqueous, pH 5–8, 37°C) to promote specific chemical reactions that can aid self-assembly, such as by reverse hydrolysis dephosphorylation.[15,26–28] Toledano *et al.* used the protease thermolysin, which links nongelling Fmoc amino acids to dipeptides via reverse-hydrolysis to form amphiphilic Fmoc-tripeptides, which then self-assemble to form a hydrogel.[26] More recently, the same group developed Fmoc-protected dipeptide amphiphiles that self-assemble to form hydrogels. The self-assembly is triggered by adding alkaline phosphatase to dephosphorylate peptide precursors, which then form hydrogels, also exhibiting antimicrobial properties.[27] Another group, Gao *et al.*, proposed the use of the oxidative enzyme tyrosinase to trigger the gel/solution phase transition of a small-molecular hydrogel of Ac-YYYY-OMe via dephosphorylation of Ac-YYYpY-OMe.[28]

*Chemical/physical crosslinked self-assembly.* Not all of SAPs have sufficient mechanical stability for *in vivo* use. Consequently, chemical and physical methods to promote intermolecular and/or intramolecular crosslinking have been investigated to enhance the mechanical strength of the resulting self-assembled structures. Ding *et al.* investigated a photocrosslinking approach using $Ru(bpy)_3Cl_2$ to link two nearby tyrosine residues resulting in dityrosine adducts and showed $10^4$-fold enhanced stiffness compared with noncrosslinked hydrogels.[29] In another study, the Chronopoulou group showed that genipin can crosslink the Fmoc-tripeptide, which itself self-assembles into a hydrogel, to enhance mechanical stiffness in a dose-dependent manner.[30]

## DISCUSSION

Self-assembled nanomaterials have been widely used in tissue engineering and regenerative medicine applications. Below we discuss a few representative examples of the use of self-assembled nanomaterials for skin wound healing. We also address several important aspects that need to be considered in developing and designing self-assembled nanomaterials.

### Self-assembled nanomaterials as wound dressings and scaffolds

Typical wound dressings are designed to physically protect wounds, maintain a moist environment, remove exudate, and allow gas exchange with ambient air.[2] Skin scaffolds, on the contrary, provide a platform where cells migrate and proliferate to reconstitute the damaged or lost tissue. Several different types of injectable hydrogels using self-assembled nanomaterials have been developed as wound dressings and scaffolds as the hydrogels exhibit high water content and allow cell proliferation in the 3D structure.[31]

Seow *et al.* developed self-assembled hydrogel dressings using crosslinked ultrashort peptides (LIVAGKC).[18] These peptides contain a hydrophobic tail with a string of amino acids that provide a gradient of hydrophobicity. The hydrophobic tail is followed by a hydrophilic headgroup to which cysteine is capped to allow disulfide crosslinking upon $H_2O_2$-mediated oxidation. The peptides self-assemble spontaneously in water to form hydrogels. Due to the disulfide crosslinks, the resulting hydrogels were significantly stiffer than noncrosslinked gels. The hydrogels also improved re-epithelialization in a full-thickness injury mouse model with no obvious sign of allergenic effects.[19] Carrejo *et al.* developed another type of hydrogel from a different type of SAP. This group used "multidomain peptides" consisting of the 16-amino acid sequence $K_2(SL)_6K_2$, which self-assemble into a nanofibrous hydrogel. The hydrogels are syringe-deliverable and have predictable degradation at the wound sites. The multidomain peptide hydrogels facilitated 3D cell culture of fibroblasts when the cells were encapsulated. Fibroblasts grew in the 3D hydrogel and created extensive networks via cell-to-cell junctions. In addition, when applied to diabetic mice with full-thickness wounds, granulation tissue and re-epithelialization formation, and wound closure were faster than groups treated with buffer only, or IntraSite, a commercially available hydrogel (Fig. 4).

Peptide amphiphiles have also been used to serve as wound dressings and scaffolds in combi-

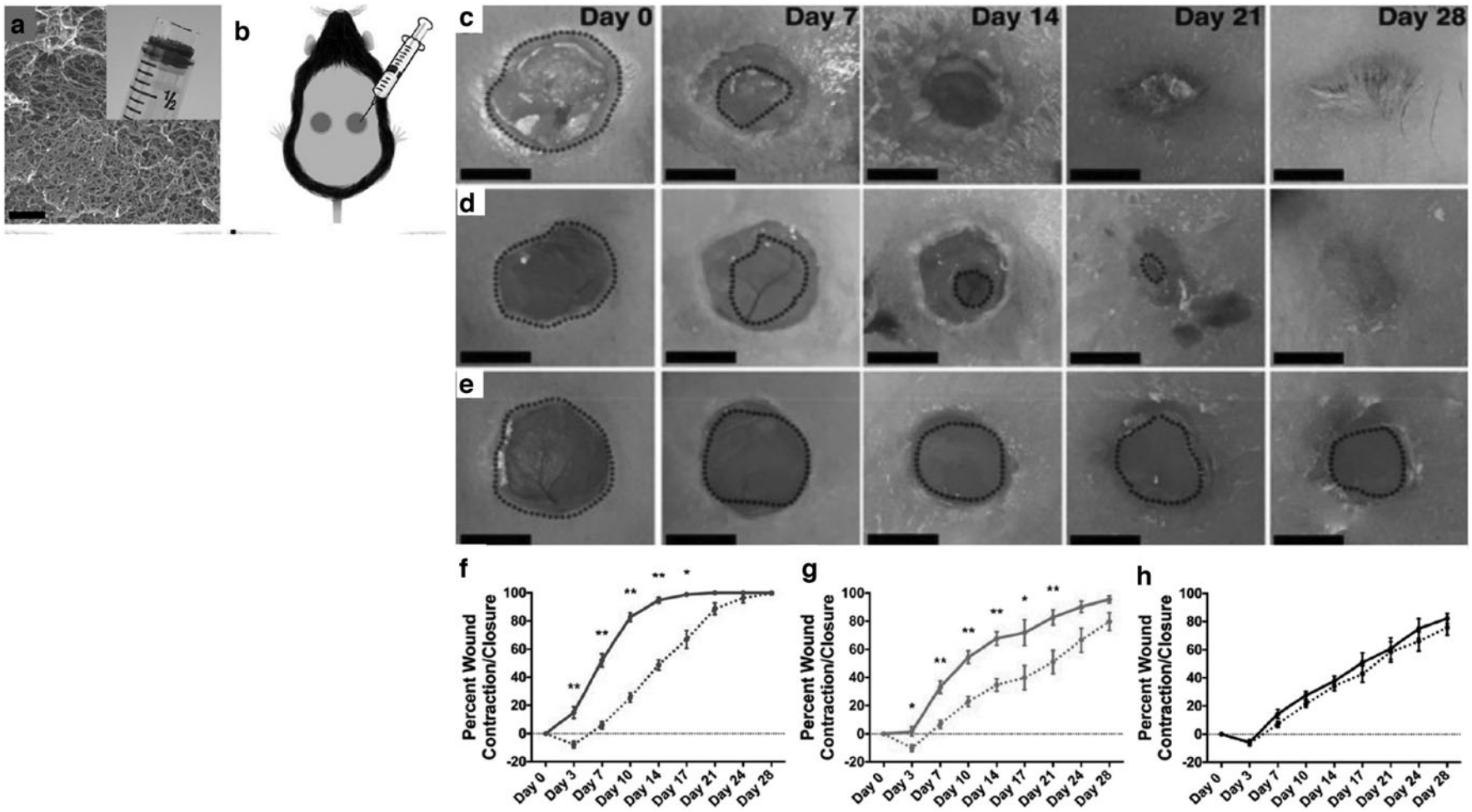


**Figure 4.** Effects of self-assembled multidomain peptides on full-thickness wounds in diabetic mice. **(a)** Nanofibers entangle and crosslink after the addition of multivalent salts, eventually forming a hydrogel (*inset*). Scale bar = 500 nm. **(b)** Because the hydrogel is syringe deliverable, it can be easily applied to wounds and conforms to their shape. **(c)** Multidomain peptide hydrogel, **(d)** IntraSite, and **(e)** buffer vehicle. Scale bars = 5 mm. Percent wound contraction (*dashed lines*) and percent wound closure (*solid lines*) for **(f)** multidomain peptide hydrogel, **(g)** IntraSite, and **(h)** buffer by normalizing to the wound area on day 0. Reprinted with permission from Ref.[19]. Copyright (2016) American Chemical Society.

nation with other molecules. One example is a heparin mimetic peptide nanofiber gel.[32] Yergoz *et al.* developed nanofiber networks formed by oppositely charged peptide amphiphiles, heparin-mimetic peptide (HM-PA, lauryl-VVAGEGD(K-psb)S-Am) and K-PA (lauryl-VVAGK-Am) in 1:2 M ratio. The mixed peptide molecules self-assembled into fibrous networks that resemble ECM.[32] These hydrogels promoted faster wound closure of full-thickness burns in mice compared with wounds covered only by commercial Tegaderm™, and control nanofibers without the heparin-mimetic motifs. In another study, also in an acute wound model, HM-PA also showed increased re-epithelialization and granulation tissue formation in full-thickness excisional wounds in rats.[33] Zhou *et al.* also used peptide amphiphile gels for burn wound healing.[34] The authors made several different types of peptide amphiphiles with a slight modification to include bioactive epitopes that mimic ECM. A cell proliferation assay was performed in thermally damaged fibroblasts and human umbilical vein endothelial cells (HUVECs) and showed a higher level of proliferation in peptide amphiphile gel-treated groups. Also, burn wounds in rats healed faster in the HM-PA-treated group where the hydrogels were modified to contain the Arg-Gly-Asp-Ser (RGDS) epitope, a well-known cell surface integrin-binding sequence.

Another interesting class of SAPs used as wound scaffolds is silk/elastin hydrogels. Silk/elastin contains repeats of silk fibroin (GAGAGS) and elastin-like (GVGVP) sequences that are recombinantly expressed.[35,36] Kawabata *et al.* proposed silk/elastin hydrogels that absorb wound exudate at physiological temperature.[35] The authors reported larger areas of granulation in wounds covered by the silk/elastin hydrogels compared with the control polyurethane film.

### Self-assembled nanomaterials for growth factor delivery

SAPs form stable hydrogels and are considered good candidates to serve as depots for delivery of bioactives to the wound. Several types of synthetic peptides have been developed and investigated to deliver growth factors and other bioactive molecules.[10,22,37–40]

One of the most widely explored SAPs is self-complementary peptides with 16 amino acids, such as RAD16-I (RADARADARADARADA), RAD16-II (RARADADARARADADA), and their derivatives with a slight modification, which self-assemble into hydrogels. Several groups have reported slow and controlled release of molecules, growth factors, and cytokines from RADA-I nanofiber scaffolds.[34,37] For example, Gelain *et al.* reported that designer SAP scaffolds made of RADA16-I and its derivatives (RADA16-DGE and RADA16-PFS) showed slow and sustained release of several cytokines.[38] In a study where the effects of the SAP hydrogels were investigated, Schneider *et al.* proposed to use SAP nanofibers containing epidermal growth factor (EGF) to accelerate wound healing.[40] They reported that EGF was preferentially released from the SAP hydrogels due to protease-mediated activity from cells in a wound created in a human skin equivalent *in vitro*. Wound closure was 3.5-fold faster and wound re-epithelialization was accelerated when treated with EGF-containing SAPs, compared with SAPs alone.

Another example is peptide amphiphiles that contain four distinct domains: (1) a bioactive molecule, (2) a polar domain that confers solubility, (3) a stabilization domain, that is, often a $\beta$-sheet forming sequence, and (4) a hydrophobic tail that enables cylindrical micelle self-assembly.[12,39] Hosseinkhani *et al.* developed injectable 3D peptide amphiphile scaffolds with encapsulated bFGF for tissue regeneration.[39] Peptide amphiphile aqueous solution was mixed with bFGF suspensions to produce injectable hydrogel scaffolds. When the bFGF and peptide amphiphile mixture was subcutaneously injected into the back of mice, a 3D hydrogel was formed *in situ*, and a significant angiogenic response was observed.

Koria *et al.* developed SAPs containing ELPs and keratinocyte growth factor (KGF) for chronic wound healing.[9] KGF was fused with 50 repeats of ELPs and the fusion proteins (KGF-ELP) were expressed in *Escherichia coli*. KGF-ELP formed nanoparticles above the transition temperature. The authors confirmed the bioactivity of KGF in the nanoparticles in an *in vitro* proliferation assay using keratinocytes. Furthermore, when KGF-ELP nanoparticles were applied to full-thickness wounds in diabetic mice, enhanced re-epithelialization and granulation were observed. More recently, Yeboah *et al.* developed another type of ELP fusion peptides containing stromal cell-derived factor-1 (SDF-1) for chronic wound healing.[22,41] The fusion protein, SDF1-ELP, self-assembled into nanoparticles at physiological temperature. When tested *in vitro*, SDF1-ELP promoted migration and vascularization of endothelial cells. Furthermore, SDF1-ELP remained intact after incubation in elastase for 12 days while free SDF1 was not detectable after incubation in the same condition (Fig. 5A–C). When SDF1-ELP in fibrin gel was applied onto *in vivo* diabetic

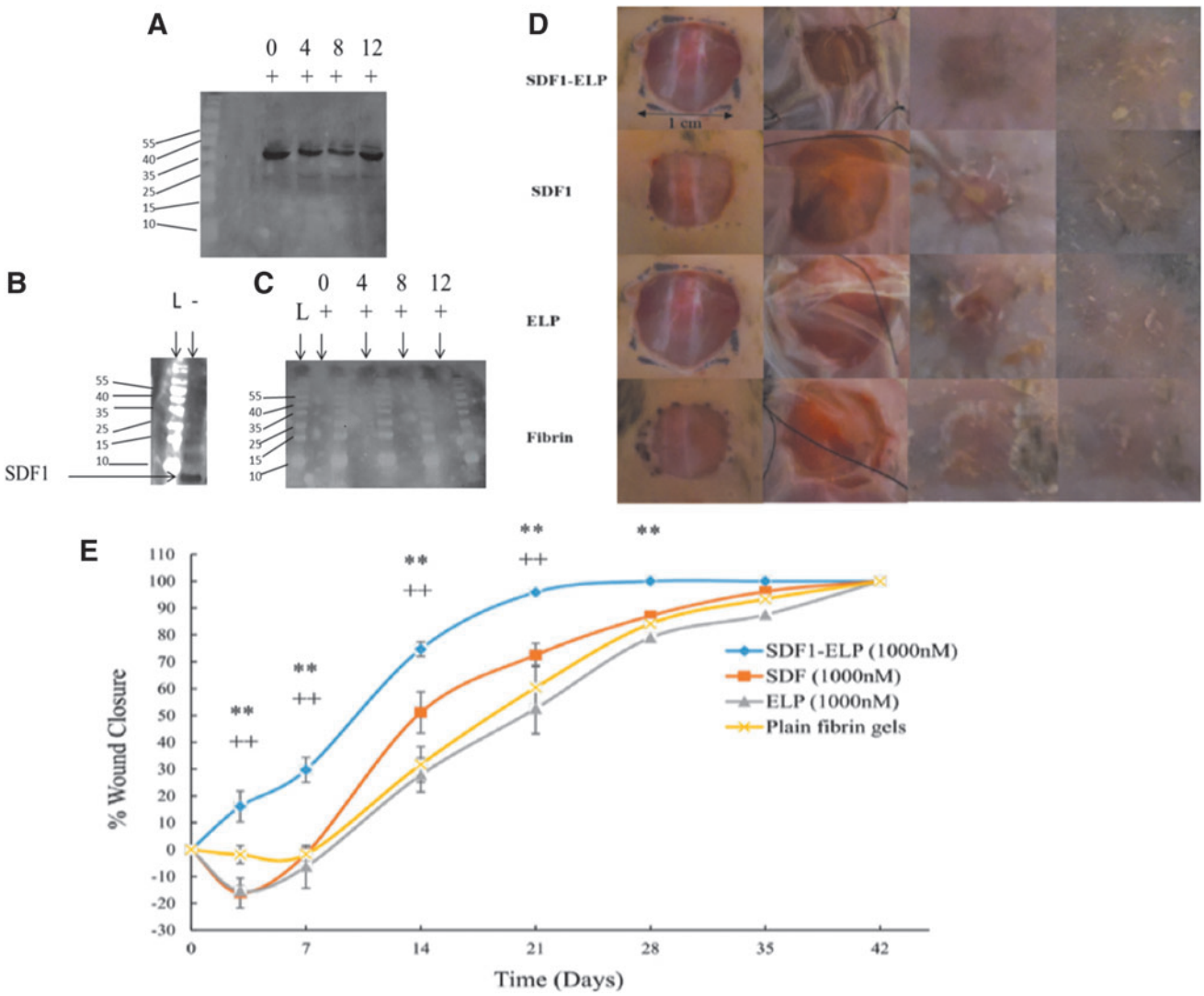


**Figure 5.** Stability of SDF1-ELP in elastase and effect of SDF1-ELP on wound healing in diabetic mice. *Left:* Degradation of SDF1-ELP or free SDF-1 by elastase. SDF1-ELP and SDF-1 were incubated in elastase over a 12-day period. Samples were pulled at 4-day intervals and subjected to Western blot analysis. **(A)** Representative blot of SDF1-ELP samples after incubation in elastase. **(B)** Lane 1, labeled L is the molecular weight ladder. Lane 2, labeled (–) is SDF-1 with no elastase. **(C)** Representative blot of SDF-1 samples in elastase. No SDF-positive bands are seen in any of the lanes. *Right:* Effect of SDF1-ELP on skin wound closure in diabetic mice. Full-thickness excisional wounds were treated with fibrin gel with SDF1-ELP particles, fibrin gel 75 containing free SDF-1, fibrin gel containing ELP particles, or plain fibrin gel (vehicle control). **(D)** Representative images of the wounds on different days. On postwounding day 28, the wound treated with SDF1-ELP was fully closed, while in the other groups it was still open, only fully closing by day 42. **(E)** Quantified wound closure as a function of time. $n=5$ (**, ++: $p<0.01$, one-way ANOVA, Fisher's LSD post-test; ++: SDF1-ELP compared with SDF1, **: SDF1-ELP compared with ELP or plain fibrin). Adapted from Ref.[41] SDF-1, stromal cell-derived factor-1.

wounds in mice, a higher number of vascular endothelial cells (CD31+ cells), faster wound closure, and much thicker epidermis and dermis were observed compared with free SDF1 and other control groups including empty ELP nanoparticles, and fibrin gel vehicle (Fig. 5D, E).

### Self-assembled nanomaterials as antimicrobials

A common complication that may make chronic wounds even more difficult to heal is bacterial infection.[1] Although conventional antimicrobial materials, such as silver, zinc oxide, and copper oxide, have proven their potential, toxicity toward human cells limits dosage and duration of application.[42] Recently, researchers have explored and developed self-assembled nanomaterials as antimicrobial agents that may prevent wounds from developing biofilms.

Chen *et al.* developed antimicrobial peptides self-assembled on gold nanodots.[42] Gold nanomaterials in general have good stability and biocompatibility, but by themselves have low antimicrobial activity. By coimmobilizing surfactin (SFT) and 1-dodecanethiol (DT), which self-assembled onto the

gold nanoparticles, the group developed SFT-/DT-Au nanodots and demonstrated antimicrobial effectiveness toward a wide range of bacterial strains, including multidrug-resistant bacteria. In another study, Lu *et al*. used gold nanoparticles coated with chicken egg white (CEW), onto which 2-mercaptomethylimidazole (MMT) molecules were self-assembled.[43] The AU@CE/MMT nanoparticles showed antibacterial effects *in vitro* and accelerated healing of full-thickness skin wounds inoculated

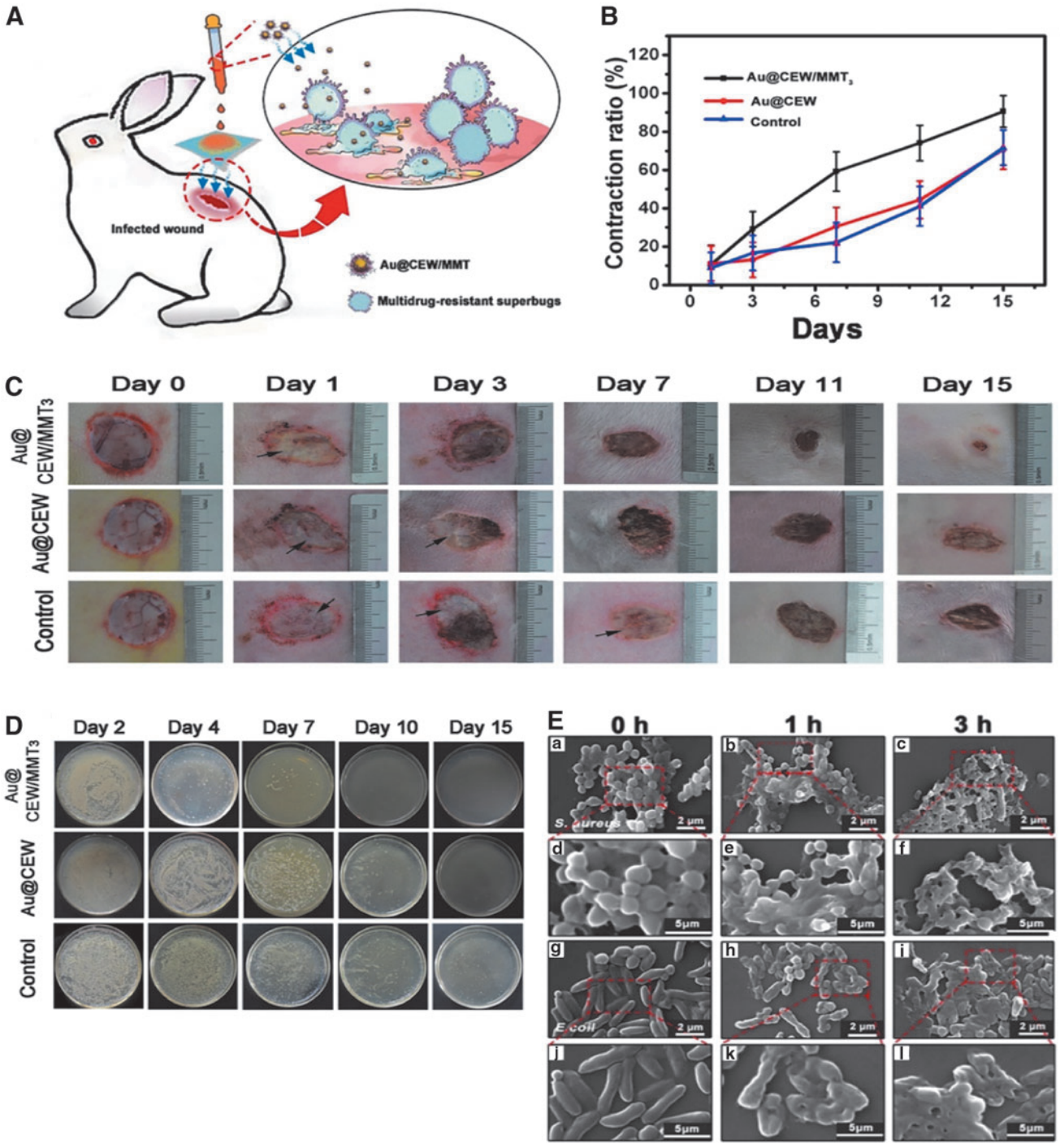


**Figure 6.** Antibacterial effects of Au@CEW/MMT on the healing of full-thickness wounds exposed to multidrug-resistant bacteria. **(A)** Schematics of experiments. **(B)** Wound contraction ratio versus time. Error bars denote the standard error of the mean ($n=3$). **(C)** Photographs of MRSA-infected wounds, either untreated or treated with Au@CEW or Au@CEW/MMT3, taken on days 0, 1, 3, 7, 11, and 15. **(D)** Photographs of bacterial incubations from wounds that were untreated or treated with Au@CEW/MMT3 or Au@CEW/MMT4. **(E)** SEM images of **(a–f)** *Staphylococcus aureus* and **(g–l)** *Escherichia coli* after treatment for 0, 1, and 3 h. Cells at 0 h displayed the typical spherical shape with a smooth and intact membrane. After 1 h of treatment, most bacteria show a blurry membrane boundary and collapsed morphology. After 3 h, the bacterial structure was thoroughly destroyed and only their debris could be observed. Adapted from Ref.[43] SEM, scanning electron microscope.

with *Staphylococcus aureus* in an *in vivo* rabbit model (Fig. 6). In the course of these studies, they also established a maximum $HAuCl_4$:MMT ratio of 1:50 that exhibited no cytotoxicity toward skin fibroblasts.

Reithofer *et al.* used hydrogels made of self-assembled ultrashort peptides, Ac-LIVAGK-$NH_2$ (Ac-$LK_6$-$NH_2$), to serve as a matrix for *in situ* silver nanoparticle synthesis.[44] The resulting Ag-Ac-$LK_6$-$NH_2$ hydrogels showed sustained release of Ag nanoparticles for up to 14 days and inhibited gram-negative and gram-positive bacteria with no significant toxicity toward human dermal fibroblasts.

Another interesting class of self-assembled nanomaterials is metallo-nucleoside hydrogels that can be self-assembled by mixing cytidine (C) with 0.5 equivalents each of $B(OH)_3$ and $AgNO_3$ (C-B-C•$Ag^+$).[31] The C-B-C•$Ag^+$ hydrogels significantly inhibited gram-negative and gram-positive bacteria *in vitro* and promoted faster wound closure of mouse burn wounds (71.15% closure compared with 33.69% in the nontreated group after 7 days of treatment).

Although not tested *in vivo*, Paladini *et al.* proposed silver-doped self-assembling di-phenylalanine hydrogels with high water content.[45] The group incorporated antimicrobial silver nanoparticles in di-phenylalanine ($F_2$)-9-fluorenylmethoxycarbonyl (Fmoc) peptides, which readily self-assemble to form hydrogels. They investigated the antibacterial effects of the hydrogels containing silver against *S. aureus* and found good antibacterial capability with 0.1 wt% of silver.

### Current challenges and future directions

Self-assembled nanomaterials offer several advantages, including precise selectivity and multifunctionality that can address specific challenges and limitations in clinic. However, long-term stability of self-assembled nanomaterials, especially peptide-based nanomaterials, is critical to the success of wound healing therapies.[46] Proteases, such as metalloproteinases and elastase, whose levels are elevated in chronic wounds, may negatively impact on the stability of peptide-based self-assembled nanomaterials.[5,7] Further efforts should be made to design protease-resistant self-assembled nanomaterials. An alternative is to design self-assembled nanomaterials that can sequester these host proteases within the wound microenvironment during the remodeling phase.

The pH of the wound microenvironment should also be considered while designing self-assembled nanomaterials for chronic wounds. While the pH in acute wounds is slightly acidic (pH4.0–pH6.3), the pH in chronic wounds is rather alkaline (pH7.15–pH10.0).[47] Therefore, development of self-assembled nanomaterials that maintain their structure even at these higher pH values is required for the treatment of chronic wounds. Understanding the impact of such environmental parameters on the ability of self-assembled nanomaterials to deliver bioactive molecules in a controlled and sustained manner, when such property is desired, is also critical.

Immunogenicity and toxicity are other major hurdles for the clinical translation of any nanomaterial. Although many of these therapies are to be used topically, the slow dynamics of chronic wound healing will likely require slow degradation and/or multiple applications thus causing prolonged exposure of the wound bed to the materials, and also the potential for significant systemic absorption. The field has progressed toward developing novel self-assembled nanomaterials with low acute toxicity; however, future studies should investigate the physicochemical properties of self-assembled nanomaterials that mitigate longer term cytotoxicity and immunoreactivity, especially in the context of skin, which is thought to be a highly immunogenic organ.[48] There is already a vast literature covering the impact of self-assembled material design on immunoreactivity, and there is considerable evidence suggesting a direct relationship between the physicochemical properties of nanomaterials and their negative effects on the immune system.[49,50] Furthermore, to proceed to clinical trials in human subjects, more relevant *in vivo* chronic wound models may be required. Currently, most *in vivo* studies rely on diabetic rodent models that exhibit delayed wound healing. Such models cannot address, in particular, the heterogeneity in human immune responses. Better animal models, perhaps such as humanized mice, may be used in the future to more thoroughly examine potential immune reactions to self-assembled nanomaterials.

Finally, self-assembled nanomaterials could help direct the remodeling phase to promote less scarring and more regenerative healing. For clinical use in humans, ideally these should eventually degrade, but the timescale of degradation should extend over several months to significantly impact remodeling. Future studies need to investigate the long-term impact of self-assembled nanomaterials on healing and scarring.

## SUMMARY

The pathophysiological mechanisms of chronic wounds are complex; therefore, multipronged approaches that address several different biological mechanisms are desirable. Self-assembled nano-

materials serve as physical scaffolds to support cell growth and migration, as well as growth factor and antimicrobial delivery systems. These nanomaterials are also relatively easy and inexpensive to manufacture and can be combined to target various aspects of the wound healing process. They can also be incorporated into existing wound dressings and combined with other treatment modalities. It should also be noted that some of the approaches reviewed in this article were so far investigated only *in vitro*, in which case, future studies will need to establish their effectiveness *in vivo*, and that none so far has been investigated in human wounds, to the best of our knowledge.

## TAKE-HOME MESSAGES

- Self-assembled nanomaterials are made of small building blocks (peptides or amphiphiles) that self-assemble to make larger complex structures, such as hydrogel scaffolds, and nanoparticles.
- Self-assembly is generally driven by noncovalent interactions, but covalent crosslinking can be introduced to stabilize structures.
- Self-assembled materials can serve as scaffolds that favor cell migration and proliferation, but also controlled release of bioactive peptides and antibiotics to the wound.
- Self-assembled materials of different types may be combined to provide multiprong therapies that address multiple wound healing mechanisms.

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### Abbreviations and Acronyms

3D = three dimensional
CEW = chicken egg white
DT = 1-dodecanethiol
ECM = extracellular matrix
EGF = epidermal growth factor
ELP = elastin-like polypeptide
FF = diphenylalanine
FFD = diphenylalanine-aspartic acid
GHK = glycine-histidine-lysine
HM-PA = heparin-mimetic peptide
HUVECs = human umbilical vein endothelial cells
KGF = keratinocyte growth factor
MMT = 2-mercapto-methylimidazole
SAP = self-assembled peptide
SDF-1 = stromal cell-derived factor-1
SEM = standard error of the mean
SFT = surfactin