Current Opinion in Colloid & Interface Science 35 (2018) 104–111

Contents lists available at ScienceDirect

Current Opinion in Colloid & Interface Science

journal homepage: www.elsevier.com/locate/cocis

Self-assembled supramolecular systems for bone engineering applications

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ARTICLE INFO

Article history:
Received 17 November 2017
Received in revised form 25 January 2018
Accepted 30 January 2018
Available online 07 February 2018

Keywords:
Self-assembly
Supramolecular materials
Osteogenic differentiation
Bone regeneration
Self-assembled peptides
Collagen
Growth factor delivery

ABSTRACT

Bone regeneration is a complicated physiological process comprising of bone formation and resorption under the circumstances of some pathological conditions. This review highlights the recent development of self-assembled supramolecular systems, including spontaneous collagen-based scaffold, self-assembled peptide-based materials, modified cyclodextrin-based materials, and assembled protein or viral particles in bone regeneration applications. These self-assembled structures can offer two unique advantages for bone tissue engineering: (1) through rational design, highly ordered self-assembled supramolecular structures can be produced to display multiple functional units in a polyvalent manner; and (2) the reversible assembly-disassembly process renders the supramolecular assembly the responsiveness towards environmental or cellular stimuli. Thus, we envision that the self-assembled supramolecular materials provide promising options in clinical bone regeneration applications.

1. Introduction

Bone regeneration is a complicated physiological process comprising of bone formation and resorption under the circumstances of some pathological conditions including osteoporosis, trauma, infection, tumor, osteogenesis imperfecta and so on [1]. Unlike other tissues, bone injuries can often heal with no scar tissue and the newly formed bone is nearly indistinguishable from the uninjured bone [2,3]. Nevertheless, in some pathological conditions bone regeneration is impaired or beyond its self-healing potential. For instance, for large bone defects due to infection, trauma, tumor resection and skeletal abnormalities, bone grafting is a common surgical procedure to augment the bone regeneration [4]. Therefore, to develop novel biomaterials to facilitate bone healing has attracted many attentions recently [5–7].

Autologous bone grafting has been recognized as the gold standard for bone graft due to non-immunogenic and good osteoinduction. Nevertheless, it is limited by insufficient supply for large segmental bone defect, donor site injuries and high infection rate [8]. Clinically, allograft bone transplantation commonly substitutes for bone transplant. However, it presents some shortcomings such as immune resistance, infection and cross-infection [9]. Therefore, synthetic biomaterials have been intensively studied for bone engineering applications. The two important concepts of bone engineering are osteoconduction and osteoinduction. Osteoconduction supports the growth of host cells into a 3D structure to form bone whereas osteoinduction is the potential to induce pluripotent cells, from nonosseous environment to differentiate into chondrocytes and/or osteoblasts, resulting in bone formation. As a result, an osteoinductive material is used to induce bone formation while an osteoconductive material could guide the bone repair [10].

An ideal bone substitute should possess no immunogenicity, weak inflammatory response and cytotoxicity, good biodegradability and excellent biocompatibility [11–14]. In particular, the mechanical strength of bone substitute must be compatible to natural bone and can sustain the operational handling and the patient's normal activities [15]. It is critical for bone substitute to be compatible with cells including preosteoblasts, osteoblasts, chondrocytes, bone marrow stromal stem cells (BMSCs) and osteoclasts. Conventional bone substitutes include bioceramics, calcium phosphate, nano hydroxyapatite (nHA), metal materials (e.g. titanium/magnesium alloy), polymers, peptides complexes and organic-inorganic hybrid composites. Due to the controllable structure and biomimetic properties, self-assembled supramolecular materials have been widely used in bone engineering applications, including bone or cartilage regeneration, the differentiation of bone related cells, drug/peptides delivery system and etc. In this article, we

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https://doi.org/10.1016/j.cocis.2018.01.015
1359-0294/

highlight the recent development of novel supramolecular assemblies in bone tissue engineering applications.

2. Collagen-based supramolecular materials in bone engineering

As the most important extracellular fibrin, collagens (COLs) provide a framework of extracellular matrices (EMC) for tissues, including vertebrate tendons, cartilage and bone in humans and mammals. Normally COLs form semicrystalline fibers in the EMC, provide tension and elasticity to cells and play an important role in cell migration and development. Among over twenty types of COLs found, type I, type II and type III COLs account for 80%~90% of the total COL content (Fig. 1A) [16–18]. Different types of COLs have different molecular structure and immunological characteristics. As the most common COL, Type I COL is secreted by osteoblasts and plays a critical role in bone growth and maintenance of normal bone structure [19]. Type II COL secreted by the chondrocytes is the main components in articular cartilage and tooth, which could effectively prevent various joint degeneration as well as promote the regeneration and repair of cartilage [20]. Type III COL is the primary fibrous COL of blood vessel and scar tissues [21].

In last decade, COL-based supramolecular materials attracted both academic and medical interests in bone regeneration, bone/cartilage replacements and drug/protein delivery owing to its good biodegradability, low antigenicity, mechanical stability, and convenient sterilization methods [22]. A major research focus was to enhance the mechanical strength of COL-based supramolecular materials for clinical applications [23–27]. For example, Levingstone et al. reported a novel construct that mimicked the inherent gradient structure of healthy osteochondral tissue [28*]. As shown in Fig. 1B, the construct includes a bone layer composed of COL (type I), an intermediate layer composed of co-assembled COL (type I) and hyaluronic acid (HyA), and a cartilaginous region composed of COL (type I and II) and HyA. The mechanical and morphological properties of these COL-based scaffolds were optimized to allow the infiltration of mesenchymal stem cells (MSCs) and the subsequent differentiation towards the required lineage *in vitro*. Combining their abilities to stimulate migration and chondrogenesis of MSCs, these COL-based supramolecular scaffolds showed great potential for promoting cartilage tissue repair (Fig. 1C) [29]. For example, it was employed to treat focal osteochondral lesions and showed an improved response in comparison to a market approved synthetic polymer scaffold composed of poly(lactide-co-glycolic acid) (PLGA) and calcium-sulfate [30].

Cui et al. developed an injectable and self-setting bone graft materials using composite COL and calcium sulfate hemihydrate [31]. This composite material, as a self-assembly mineralized fibril, has the same characteristics as natural bone in both major hierarchical structure

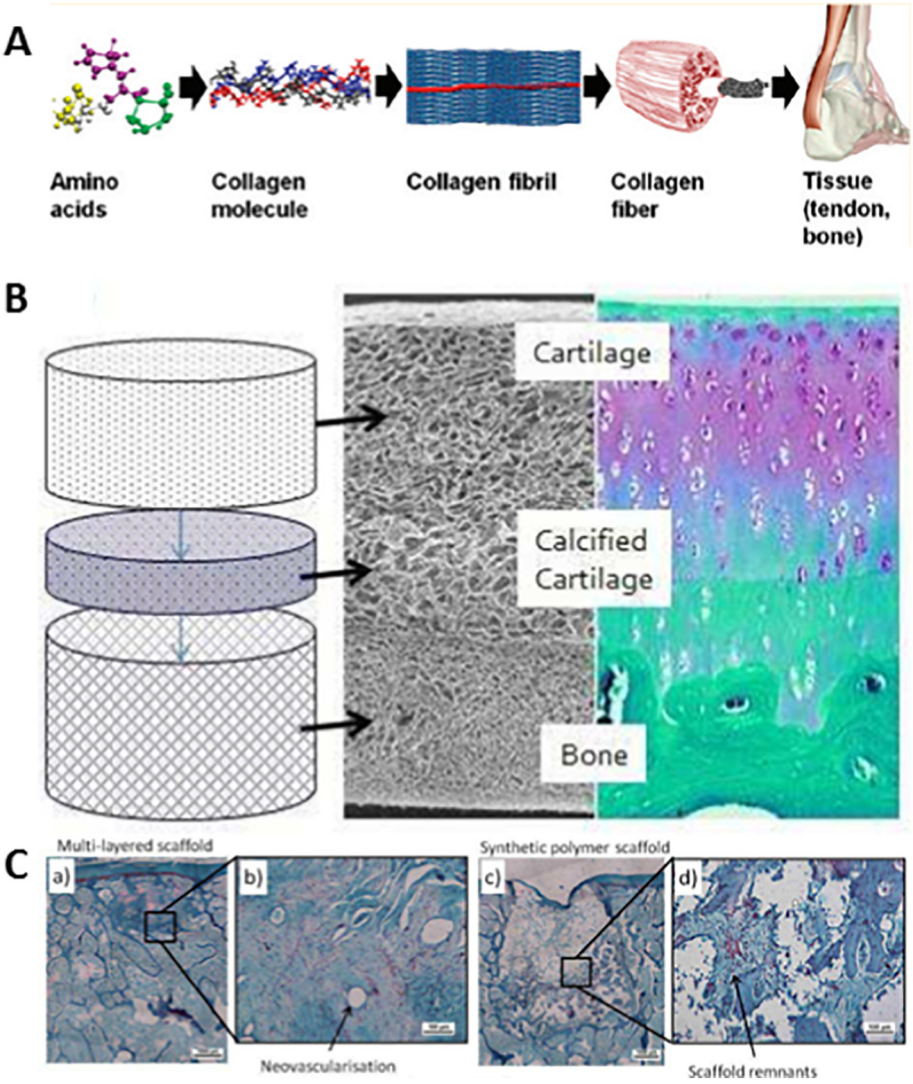

Fig. 1. (A) Hierarchical structure of collagen protein materials. (B) Scheme of multi-layered COL-based scaffolds implanted in the goat stifle joint defect site. (C) Histological analysis of tissue formed in the multi-layered scaffold and synthetic polymer scaffold groups. Panels (A) is reproduced with permission from Ref [18]; copyright 2011 American Chemical Society. Panels (B–C) are reproduced with permission from Ref [30]; copyright 2016 Elsevier.

and composition. The *in vitro* and *in vivo* histology studies demonstrated that such composite could stimulate the cell adhesion and proliferation, resulting in an accelerated bone formation. In addition, many research groups have reported the incorporation of bone growth factors in COL via supramolecular interactions. For example, Matsiko et al. demonstrated that the hybridization of COL with HyA could form a scaffold that could encapsulate factor TGF-β3, a critical growth factor to enhance the chondrogenic differentiation of MSCs, and eventually lead to subsequent cartilage-like matrix deposition [32•]. In another study, Stupp et al. reported the incorporation of bone morphogenetic protein-2 (BMP-2) into a COL-based supramolecular nanofiber system [22], which could enhance the bioactivity of BMP-2 and therefore lead to effective bone regeneration *in vivo*.

3. Synthetic peptide based supramolecular materials in bone regeneration

Diverse classes of peptides have been employed to mimic natural tissue fibrous structures of ECM for 3D cell-material co-culturing and tissue engineering applications [33]. Among them, peptide based supramolecular materials are the most widely studied topic in bone regeneration [34]. The self-assembled peptides mainly include short peptides and peptide derivatives, glycopeptides, peptide amphiphiles, self-complementary ionic peptides, and hairpin peptides, etc. [35]. By control of the supramolecular interactions, peptides or peptide derivatives can assemble to give different hierarchical structures, which place functional groups including carboxylate, amine, carbohydrates, metal chelators or cell-binding motifs in a spatially organized manner. Therefore, such assemblies can mimic the ECM and modulate the adhesion, proliferation and differentiation of MSCs, eventually, resulting in bone tissue regenerations [36].

3.1. De novo designed peptide amphiphiles

Stupp and his coworkers pioneered in using peptide amphiphiles to form nanostructured supramolecular gels to promote the mineralization and crystallization of nHA in a biomimetic microenvironment (Fig. 2) [37••]. The peptide amphiphile contains a phosphoryl group to induce the formation of calcium phosphate minerals, an Arg-Gly-Asp (RGD) sequence to enhance cell adhesion, and a long alkyl chain to the *N*-terminus of the peptide to mediate the assembly process. Such peptide amphiphile could form discrete nanofibers via a pH-controlled and reversible process, which could direct mineralization of nHA to form a composite material that showed similar structural features as native bones. In a further study with the rat femoral large-size defect model, *in vivo* micro-computed tomography and histology results showed that peptide amphiphiles based nanostructured gels with embedded phosphoryl groups could promote new bone formation, showing a superior effect in comparison to peptide amphiphile gels without phosphoryl groups or allogenic bone matrix used in clinic [38]. In another study, a selected peptide-amphiphile was self-assembled into high-aspect ratio nanofibers to display bioactive peptide epitopes along the periphery of nanofiber in physiological conditions. This paper highlights the influence of self-organized structure and appearance of scaffolds to cell adhesiveness. The coating of linear and branched PA with clear surface retention onto traditional poly(glycolic acid) (PGA) scaffolds could significantly enhance cell adhesion and phenotype expression under *in vitro* cell culture conditions [39].

3.2. Self-complementary peptide

Zhang and coworkers first identified a self-complementary peptide sequence, i.e. $(Ala\text{-}Glu\text{-}Ala\text{-}Glu\text{-}Ala\text{-}Lys\text{-}Ala\text{-}Lys)_2$ (EAK16), which alternates hydrophobic and hydrophilic residues, from zuotin, a yeast protein that can bind preferentially to left-handed Z-DNA [40], and discovered that EAK16 could form an insoluble aggregate *in situ* [41]. An analogous ionic self-complementary peptide, RAD16, in which arginines substitute for lysines and aspartic acids substitute for glutamic acids, was designed, which could form hydrogel-like matrix in a physiological solution and support the anchoring and growth of mammalian cells [42••]. This supramolecular assembly of RAD16 peptide, also branded as *PuraMatrix™*, has excellent stickiness, fluidity and plasticity abilities, which can be used to mimic the hierarchical structure of major ECM components and used in bone tissue regeneration [43–46]. For example, *PuraMatrix™* could promote a rapid hemostasis and accelerate new bone regeneration in the ilium bone defect model of New Zealand

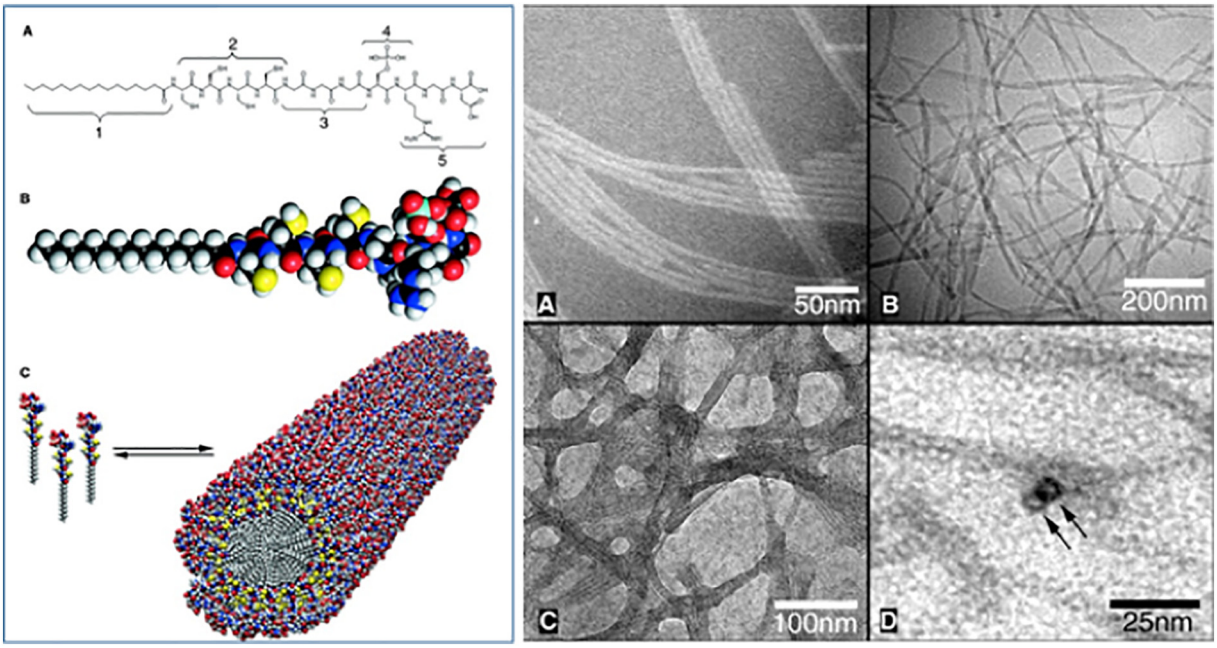

Fig. 2. (***Left*** Panel) (A) Chemical structure of the peptide amphiphile, highlighting the cell adhesion peptide motif and the phosphorylated serine residue for calcium binding. (B) Molecular model of the peptide and (C) the schematic illustration of the peptide assembly. (***Right*** Panel) TEM analysis of the self-assembled nanofibers before crosslinking (A, B) and after crosslinking (C, D). Figures are reproduced with permission from Ref. [37••]; copyright 2001 American Association for the Advancement of Science.

rabbits [47]. Radiographic analysis and histological test emphasized that the RAD16 nanofiber could efficiently promote bone regeneration and exhibited very good biocompatibility. As comparison, bone wax inhibited osteogenesis and gave rise to severe inflammatory response *in vivo* [47].

Like natural ECM, *PuraMatrix™*, can incorporate a variety of cytokines and growth factors due to its highly porous structure. As a result, *PuraMatrix™* could maintain the differentiation potential of MSCs, and promote then new bone formation in a 3D structure. Moreover, *PuraMatrix™* with MSCs and platelet-rich plasma (PRP) could tune cell behaviors and exhibit excellent osteoinductive capacity, therefore it can serve as a promising bone tissue engineering material for a clinical setting rather than traditional grafting approach [48]. Furthermore, based on the reproducible and customizable capacity of RAD16 peptide, a self-assembling RAD16 scaffold doping with chondroitin sulfate (ChS) or decorin were designed via noncovalent interactions to mimic the native cartilage ECM [49]. *In vitro* assessments demonstrated that the RAD16 based scaffolds could modulate the re-differentiation of human articular chondrocytes and induce human adipose derived stem cells (ADSCs) to differentiate into chondrocytes. Hence, it is a potential 3D culture system suitable for promoting chondrogenic differentiation of stem cells [49].

Hybridized RAD16 based biomimetic scaffold composed of heparin sodium salt was developed to study the effect of heparin moieties on unique binding and release of growth factors such as VEGF [50]. Interestingly, Western Blot and real-time PCR results demonstrated that the RAD16 scaffold decorated with heparin moieties could effectively enhance the cell bioactivity by modulating the expression of some specific chondrogenic markers and chondrogenic conversion of ADSCs [46,51]. In another study, RAD16 peptide was derivatized with biologically active motifs such as osteogenic growth peptide ALK (ALKRQGRTLYGF), osteopontin cell adhesion motif DGR (DGRGDSVAYG), bone-cell secreted-signal peptide and RGD-based cell binding sequence PGR (PRGDSGYRGDS). In comparison to pure RAD16 motif, the novel hybridized RAD16 scaffolds could significantly promote the proliferation and osteogenic differentiation of mouse pre-osteoblast MC3T3-E1 cells [45].

To study the bone-related cellular interactions with self-complementary peptide nanofibers and the subsequent effects on cellular responses, other similar systems were also reported. For example, KLD12 ($[KLDL]_3$) peptide was synthesized and compared with RAD16 in promoting the chondrogenesis of BMSCs [52]. FEFEFKFK peptide was synthesized that can self-assemble into anti-parallel β-sheets nanofibers, mimicking the native ECM microenvironment [53]. When the FEFEFKFK hydrogels were used as nucleus pulposus cell carrier for intervertebral disc tissue engineering, the upregulation of nucleus pulposus-specific genes (KRT8, KRT18, and FOXF1) were observed, which could restore the nucleus pulposus phenotype along with monolayer dedifferentiation. In addition, type II COL and aggrecan deposition increased in a time-dependent manner modulated by the FEFEFKFK peptide hydrogel. This system can be potentially used in cell-based therapy for nucleus pulposus tissue engineering [54].

3.3. Other ECM-mimetic assemblies

One major direction of using peptide-based assemblies in bone tissue engineering is to mimic the fibrous structures like the major ECM components [55]. Glycosaminoglycans (GAGs) and glycoproteins (PGs) are critical components of EMC, which not only directly induce cell adhesion, proliferation and differentiation, but also provide effective lubrication and protection for the joints. Notably, GAG have several types including dermatan sulfate (DS), HyA, chondroitin 4-sulfate (ChS-4) and chondroitin 6-sulfate (ChS-6), decorin. As reported, a GAG-based nanofiber system could be produced via self-assembly to imitate the ingredient combinations, specific structure and bioactivities of natural HyA, as the major component of articular cartilage [56]. *In vitro* results showed that such glycopeptide nanofibers could effectively promote chondrogenic differentiation of MSCs. Moreover, *in vivo* results revealed that the nanofibers could induce the chondrogenesis of MSCs and cartilage regeneration without exogenous growth factors. These results highlighted that self-assembled nanofibers could be potentially used in MSCs-based cartilage regeneration therapies [56].

It is widely accepted that the destructed extensive articular cartilage is hard to self-repair without the supply of nerves and blood vessels. Surprisingly, when a novel bioactive hydrogel with 3D nanofibrillar networks was formed via the self-assembly of COL mimetic peptides, which could lead to chondrogenic differentiation and cartilage regeneration [57]. The results showed that chondrogenic differentiation *in situ* and chondral EMC deposition were significantly increased with the endogenous secretion of TGF-β1. Hence, this COL-based hydrogel could induce BMSCs differentiation in situ and improve the efficiency of cartilaginous tissue regeneration [57]. Similarly, self-assembling COL-like peptides with gold and silicon surfaces were synthesized to evaluate native and mutation states related to osteogenesis imperfecta. It showed that the hydrophilic amino acid within the supramolecular assembly could accelerate the severity of disease, which promoted the trend of this structural organization change and influenced the nature of HA mineralization pattern after native mutations of osteogenesis imperfecta [58].

4. Supramolecular system for controlled delivery in bone tissue engineering

4.1. Self-assembled supramolecular system for bone growth factors delivery

Cellular behaviors and ultimate tissue responses were modulated by various morphogenetic signaling pathways in osteogenesis. Among all growth factors, BMP-2 has received tremendous attention due to its effective bone induction. However, its clinical application is limited because of its susceptibility to deactivation and degradation by physiological microenvironments [59•]. Many supramolecular assemblies have been developed for the controlled delivery of BMP-2. For example, negatively charged heparin doping with BMP-2 can form polyelectrolyte system that prevent deactivation and further enhance the osteoinductive capability of BMP-2 [60]. Furthermore, *in vitro* results indicated that sulfonated polyrotaxanes/BMP-2 composites improved MC3T3-E1 cell viability and mineralized matrix deposition of nHA compare to pure BMP-2 or heparin/BMP-2 composites. Therefore, this system provides an interesting strategy for the delivery of BMP-2 for a better osteoinduction in clinical bone regeneration [60].

Self-assembled peptide based materials were commonly employed to deliver recombinant human bone morphogenetic proteins such as BMP-2, TGF-β1, angiogenic basic fibroblast growth factor (bFGF) and/or to embed BMSCs for cartilage bone tissue regeneration [61–63]. Stupp and coworkers reported a self-assembled peptide amphiphile scaffold that includes a TGF-β1binding peptide motif (Fig. 3-left). The *in vitro* results showed that the scaffold could effectively maintain the hMSC viability and enhance the regenerative potential of chondral defects *in vivo*. With mild and facile injection into the joint *in vitro*, the scaffolds promoted the chondrogenic differentiation of hMSCs and articular cartilage regeneration. The *in vivo* data also supported that the designed supramolecular nanofibers could significantly exert biological responses by increasing the integration of materials with surrounding cartilage and/or subchondral bone, and hence improve the cartilage regeneration (Fig. 3-right) [64••].

An elegant study reported by Grodzinsky and coworkers highlighted the significance of reversible supramolecular interactions in the controlled release of TGF-β1 for promoting chondrogenesis of BMSCs [65•]. Three parallel TGF-β1 delivery systems were studied: (1) TGF-β1 was tethered to self-assembling peptide hydrogel by strong biotin-streptavidin binding (Teth-TGF); (2) TGF-β1-binding peptide was incorporated in the peptide sequence to adsorb TGF-β1 (Ads-TGF); and (3) agarose hydrogels were used in the presence of TGF-β1

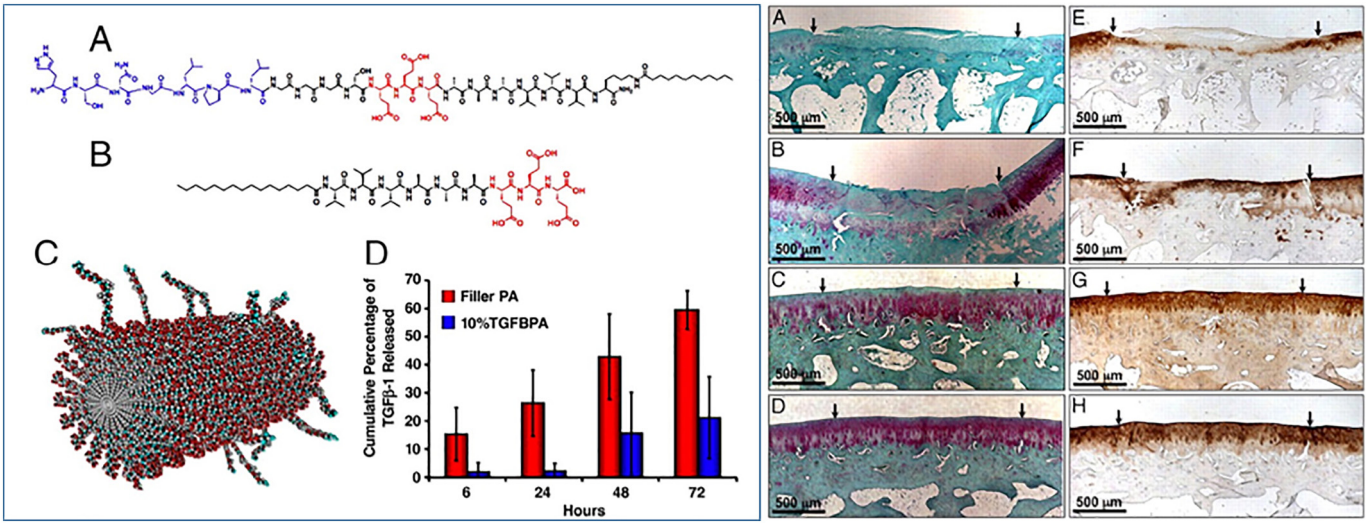

Fig. 3. (***Left*** panel) Chemical structure of (*A*) TGF-binding peptide amphiphile (TGFBPA) and (*B*) filler peptide amphiphile (filler PA). (*C*) Schematic illustration of assembly of the mixed TGF-binding and filler peptide amphiphiles. The binding epitopes are exposed on the surface of the nanofiber. (*D*) ELISA results showing TGF-β1 release from filler PA and 10 mol% TGFBPA. (***Right*** panel) Histological sections of articular cartilage defects 12 weeks after treatment, with safranin-O staining for glycosaminoglycans (A–D) and type II COL staining (E-H). The cartilage defects were treated with (*A*, *E*) 100 ng/mL TGF-β1, (*B*, *F*) filler PA + 100 ng/mL TGF-β1, (*C*, *G*) 10%TGFBPA +100 ng/mL TGF-β1, and (*D*, *H*) 10%TGFBPA alone. This is reproduced with permission from Ref [64••]; copyright 2010 National Academy of Sciences of the United States of America.

(Med-TGF). The results shown that Ads-TGF could promote chondrogenesis of BMSCs via modulating cell proliferation and cartilage-like ECM accumulation, whereas Teth-TGF did not induce chondrogenesis. On the other hand, Ads-TGF hydrogels could modulate the full-length aggrecan production by BMSCs, while TGF-β1-supplemented medium with agarose hydrogels merely affected the formation of aggrecan cleavage product. Moreover, phosphorylated Smad2/3 was detected as early as 4 days in Ads-TGF hydrogels, when Med-TGF hydrogels needed 3 weeks [65•]. Taken together, these results revealed that the dose and signal duration of TGF-β1 played a crucial role in minimizing aggrecan cleavage product, while the self-assembling peptide systems are advantageous for the controlled release of growth factors like TGF-β1 [65•,66].

In addition, RAD16-I peptide assemblies (*PuraMatrix™*) has also been used in the delivery of different kinds of growth factors for bone engineering. For example, *PuraMatrix™* could incorporate rhBMP-2 and serve as a scaffold to enhance bone regeneration in a rabbit bone augmentation model [67]. In another study, the released basic fibroblast growth factor (bFGF) from the designed RAD16 peptides could effectively promote the proliferation of NIH-3 T3 cells and activated downstream ERK signaling pathway [68]. RAD16-I could self-assemble with HyA to form composite hydrogels (RAD-HyA), which were applied to control the release of recombinant adeno-associated virus vectors (rAAV) so as to genetically modify primary hMSCs. Results showed that the RAD-HyA systems significantly controlled sustained release behavior of rAAV vectors and promoted the effective transduction (up to 80%) and chondrogenic differentiation (up to 21 days) of hMSCs. This study implied that RAD16-I peptide could be a promising material to control the release manners of rAAV vectors for further applications in bone reengineering [69].

4.2. Cyclodextrin based supramolecular materials for hydrophobic drug delivery

Cyclodextrins (CDs) are a family of natural cyclic oligosaccharide with a hydrophilic external surface and a hydrophobic central cavity. Three different CDs are the most commonly used in chemistry and drug delivery because of their excellent biocompatibility, low toxicity and special molecular recognition mechanism. In particular, the hydrophobic internal cavity makes them ideal delivery systems for hydrophobic drugs via the host-guest supramolecular interactions [70].

Statins such as simvastatin (SV) are a kind of representative hydrophobic drugs, which could induce bone regeneration but suffer from low water solubility. Using hydroxypropyl and methyl modified β-CD (HP-β-CD, RM-β-CD), SV could be loaded within the internal cavity through the inclusion complexation mechanism, which greatly improved its water-solubility, drug release profile and the effect in promoting the osteogenic differentiation efficiency of MC3T3-E1 cells [71]. Similarly, poloxamine (Tetronic® 908) was assembled with α-CD to form supramolecular hydrogels (T-CD) that could load with SV and/or BMP-2. The results showed such T-CD supramolecular scaffolds could significantly improve the bone repair with at the graft site [72]. In addition, it showed a better release profile of both SV and BMP-2 than using poloxamine gel alone. α-CD could also assemble with polysaccharide chondroitin sulfate (ChS) or HyA and form supramolecular gels in the presence of Pluronic® F68 (PF68) [73]. Both ChS/PF68/α-CD and HyA/PF68/α-CD gels were employed to deliver rAAV vectors to hMSCs, which could induce higher rAAV local concentrations, more long-terming release of transgene expressions, as well as enhanced cellular viscoelasticity in comparison to original gels. Moreover, the addition of HyA significantly increased the bioactivity and cytocompatibility of those gels based on poloxamine (Tetronic® 908) [73].

In another study, β-CD nanoparticles were prepared by the supramolecular assembly of amino- and histidinyl-modified amphiphilic β-CDs. Upon loading with a hydrophobic oligopeptides drug (an osteoclast inhibitor), these β-CD nanoparticles could pass through the membrane of bone marrow-derived macrophages (BMMs) [74]. The loading capacity of the drug was close to 98% and *in vitro* results demonstrated that the drug-loaded nanoparticles showed excellent cellular uptake and viability. It was presumed that the permeability of intracellular oligopeptide into BMMs were closely associated with the macropinocytosis pathway, and the supramolecular interactions between the β-CD vector and the oligopeptide dictate the delivery profile [74].

4.3. Self-assembled drugs for bone diseases

To improve the stability and bioavailability, small molecular drugs could self-assemble via supramolecular interactions to improve the administration profile. Salmon calcitonin (sCT), a therapeutic polypeptide, is generally applied for bone diseases. However, it has short half-life and needs frequent injection. A self-assembled sCT and dipeptide (Asp-Phe, DF) supramolecular nanoparticles could effectively prolong the

bioavailability of sCT to more than 30 days after subcutaneous injection. The self-assembled and releasing mechanisms were thoroughly investigated through *in vitro*/*vivo* studies and molecular dynamics simulations [75]. For most osteoporosis patients, bisphosphonate (BP) has been confirmed to be an effective bone resorption inhibitor and widely used in clinical application. Two drugs, pamidronate (Pami) and alendronate (Alen), were derivatized with a dipeptide Fmoc-Phe-Phe, a known hydrogelator. The derivatives self-assembled into nanofibers which could form supramolecular hydrogels promoted by an acidic microenvironment [76]. *In vitro* results showed both assemblies inhibit the osteoclastogenesis of BMM cells. Since the bone defect sites are generally more acidic, these *in situ* formed hydrogels could effectively increase the local drug concentration at the bone resorption lacunae *in vivo*, therefore lead to a more effective treatment of osteoporosis [77].

5. Virus-based scaffolds

A key structural feature of bone tissue is the hierarchically organized structure spanning over a broad range of length scale, i.e. from a few nanometers to tens of centimeters. While supramolecular interactions are the "mortars", robust nano-size building blocks are needed as the "bricks" to such kind of scaffolding materials. Recently, viruses have used as enabling platforms for different applications, including in the field of biomaterials and tissue engineering [77]. Simple viruses represented monodispersed supramolecular assemblies with organized 3D structure along with batch-to-batch consistency (Fig. 4a). It could be reengineered to present biological signals for cell functions via chemical conjugation and genetic modification (Fig. 4d, e). The multivalent coat proteins could be derivatized with functional ligands to enhance the cell-material interactions (Fig. 4b) [78]. Because of these specific properties, viral nanoparticles have been considered as suitable candidates for ligand displaying and ECM mimicking.

Wang and coworkers reported that the self-assembling virus-based 2D and 3D biomaterials could support cell growth and modulate cellular behaviors such as adhesion, proliferation, migration, and differentiation. In particular, when BMSCs were cultured on different virus films, a saturated up-regulation of osteo-specific genes (osteocalcin, osteopontin and osteonectin) at day 14 was observed, which was 7 days earlier than that of control studies [79,80]. In addition, BMSCs showed high calcium depositions stained by alizarin red at 14 days [81]. The accelerated osteogenesis could be attributed to the early onset of BMP-2 expression, modulated by the nanotopographical feature of viral assemblies [82••].

The monodispersity of virus nanoparticles makes it easy to generate organized 1D, 2D, or 3D assemblies (Fig. 4c). For example, engineered phage films were prepared to study the promotion of osteogenic differentiation of MSCs [83]. When cultured in osteogenic media for 2 weeks, higher expression levels of osteocalcin and osteopontin were observed in MSCs seeded on the wild type and mutated bacteriophage M13 films compared to poly-lysine film. MSCs grown on the phage film aggregated to form nodule-like structures, which would attract calcium deposition and mineralization. Gene expression analysis confirmed that MSCs on both WT and engineered-phage films showed

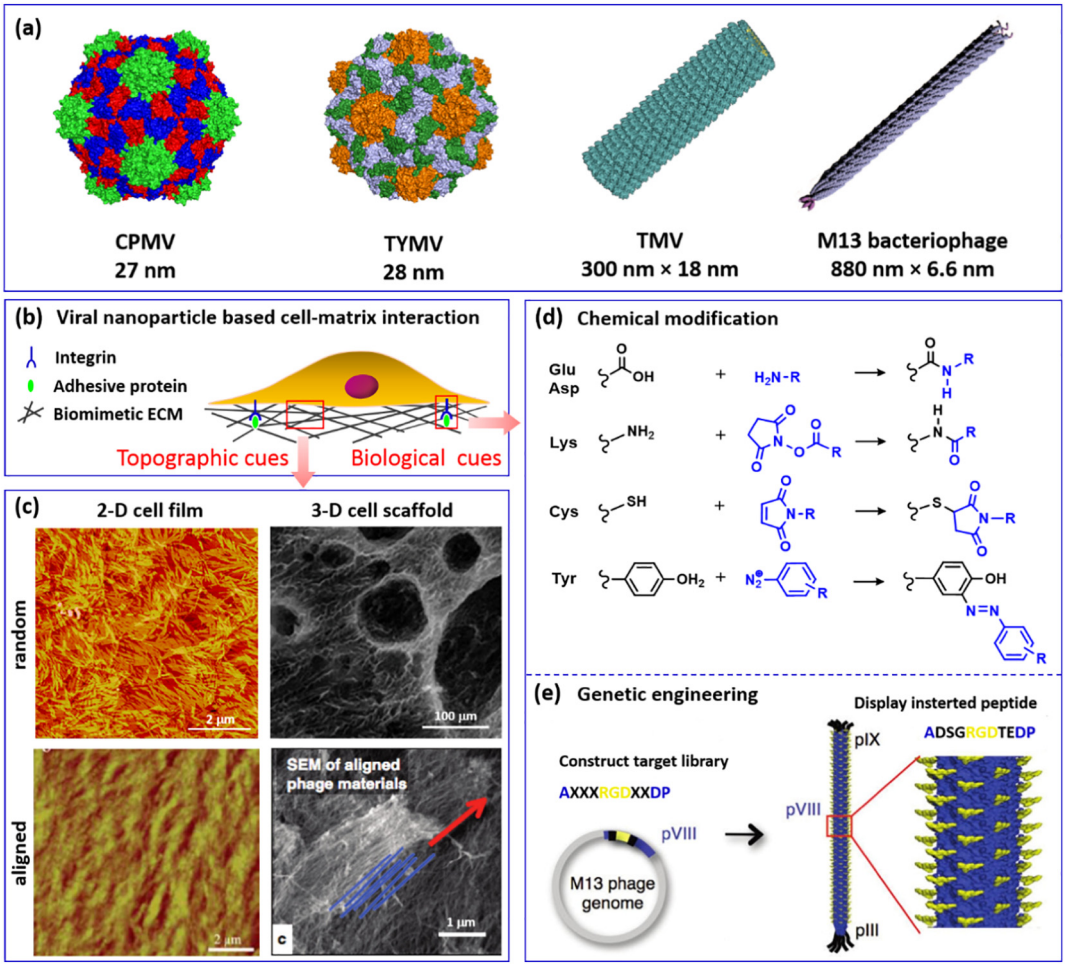

Fig. 4. (a) Typical viruses that have been used for tissue engineering. CPMV: cowpea mosaic virus; TYMV: turnip yellow mosaic virus; TMV: tobacco mosaic virus; M13 bacteriophage. (b) Schematic illustration of cell-matrix interactions. (c) AFM and SEM of virus-based 2D films and 3D scaffolds with random and aligned structures for cell supporting. (d) Typical chemical reactions to modify viral capsids. (e) Examples of genetic engineering strategy to insert peptide sequences on the capsid of M13 phage. The figure is reproduced with permission from ref. [78]; copyright 2015 Wiley.

significantly higher up-regulation of osteoblast gene expression than control systems [84]. Furthermore, MSCs on phage films have 1.5 times more calcium-containing matrix cells than films without phage. Therefore, the phage films can induce the differentiation and mineralization of MSCs in osteogenic media. Further research reported the display of peptides RGD and PHSRN on M13 and investigated the effect of biological cues on the osteoblastic differentiation of MSCs [85•]. The results highlighted that polyvalency and unique structural features of different virus particles offer a powerful supramolecular system for bone tissue engineering applications.

6. Conclusion and perspective

A few classes of typical supramolecular materials used for bone tissue engineering applications are reviewed in this article. Apparently, the supramolecular structures, resulting from the assembly of natural proteins or protein particles (like virus nanoparticles), peptides and peptide derivatives, as well as cyclodextrin and other saccharides-based building blocks, can offer two unique advantages for tissue engineering in general and for bone tissue engineering in specific: (1) through rational design, highly ordered self-assembled supramolecular structures can be produced to display multiple functional units in a polyvalent manner; and (2) the revisable assembly-disassembly process renders the supramolecular assembly the responsiveness towards environmental or cellular stimuli. Combined these two features, supramolecular assemblies have been widely employed in different kinds of bone tissue engineering applications. An important future endeavor of this exciting research field is to explore the clinic potentials of such materials. To achieve this, more careful studies are needed in terms of the investigation of the *in vivo* biocompatibility and pharmacokinetics of different materials. In addition, to match the mechanical strength of natural bones, supramolecular assemblies will play a pivotal role in the development of composites of inorganic or polymeric materials tailored with different kinds of cell-affinity units.

Acknowledgements

JS's work was supported by the National Natural Science Foundation of China (81771491), the Outstanding Talents Cultivation Program of Shanghai Health System (2017BR011), and the Shanghai Municipal Science and Technology Commission Key Program (15411950600). QW would like to acknowledge the partial financial support from the NSF ECCS-1509076 and NSF OIA-1655740.

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