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HYDROGEL-BASED NANOCOMPOSITES OF THERAPEUTIC PROTEINS FOR TISSUE REPAIR

Suwei Zhu¹ and Tatiana Segura^{1,*}

¹Chemical & Biomolecular Engineering Department, University of California Los Angeles, 420 Westwood Plaza, 5532-C Boelter Hall, Los Angeles, CA 90095, USA

Abstract

The ability to design artificial extracellular matrices as cell instructive scaffolds has opened the door to technologies capable of studying cell fates *in vitro* and to guide tissue repair *in vivo*. One main component of the design of artificial extracellular matrices is the incorporation of protein-based biochemical cues to guide cell phenotypes and multicellular organizations. However, promoting the long-term bioactivity, controlling the bioavailability and understanding how the physical presentations of these proteins impacts cellular fates are among the challenges of the field. Nanotechnology has advanced to meet the challenges of protein therapeutics. For example, the approaches to incorporating proteins into tissue repairing scaffolds have ranged from bulk encapsulations to smart nanodepots that protect proteins from degradations and allow opportunities for controlled release.

Introduction

Biomaterials for tissue repair create a permissive environment to promote tissue formation *in vivo* or to study cell differentiation, morphogenesis and cell fate decisions *in vitro*. The delivery of proteins is an ideal approach to promote and guide these processes, however, delivery of proteins poses challenges due to their low stability in serum and the need to control their temporal spatial bioavailability to match the need of the tissue or differentiation state of the cell. In the natural extracellular matrix (ECM), stability as well as bioavailability of proteins can be controlled by the extracellular matrix environment. For example, heparan sulphate, a component of the ECM, can bind growth factors with a high affinity resulting in their immobilization; this binding not only enhances the stability of growth factors but also modulates the fate and phenotype determination of nearby cells [1,2]. Elegant experiments temporally controlling the bioavailability and dose of vascular endothelial growth factor have demonstrated the delicate balance between appropriate dosing and time of release with therapeutic outcome in muscle as well as bone regenerations strategies [3,4]. Although

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*Corresponding author: Dr. Tatiana Segura, tsegura@ucla.edu, phone: 1 (310) 206 3980, postal address: 420 Westwood Plaza, 5532-C Boelter Hall, Los Angeles, CA 90095, USA.

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single protein delivery of basic fibroblast growth factor strongly stimulates cell growth, matrix production and fracture repair [5], most therapeutic and differentiation applications require the orchestrated delivery of multiple proteins. Thus, the challenge is to engineer systems to orchestrate the delivery of multiple proteins while promoting the long-term activity and bioavailability of the proteins. Furthermore, understanding how the physical presentation and cooperative/synergistic association of these proteins, as well as how the topology and adhesiveness of the underlying matrices can impact cellular fates, may hold the key to favorable therapeutic outcomes.. Initial approaches have utilized polymeric scaffolds to vary the temporal availability of growth factors [6], recombinant engineering to create hybrid modalities [7] and synergistic association [8•]. In this review we will begin by looking at recent clinical trials on using growth factors for tissue repair (Table 1). Next we will elaborate on the critical issues in protein therapy (protein stability, bioavailability, multivalency, delivery, and presentation) and focus on current advances in using protein-based nanomaterials to overcome these limitations (summarized in Figure 1). At the end of the review, we give an overview of other protein nanomaterials that are not currently used in the applications of tissue repair but that we feel could provide unique characteristics in tissue regeneration.

Approaches to increase long-term activity

Widely used in polymer conjugation, the attachment of poly(ethylene glycol) to proteins, termed PEGylation, improves the stability and lowers the immunogenicity of modified proteins, yet preserving the bioactivity for the long-term is still challenging with most random PEGylation approaches. Strategies to introduce polymerization sites at specific sites within a protein have been reported. Specific N-terminal amine modification based on its different pKa from the lysines on the side chains of protein surfaces was used for *in situ* atom transfer radical polymerization (ATRP) of PEG-like conjugates [9]. In an alternative approach, posttranslational protein splicing was used to insert an intein, the cleavage of which would provide a thioester moiety for the initiation of ATRP on the C-terminus of proteins to also polymerize a PEG-like polymer [10•]. Intravenous injection of these conjugated proteins showed a 15- to 40-fold increase in their blood exposure than the unmodified proteins, and demonstrated tumor accumulation via the enhanced permeability and retention effect [10]. Beyond the scope of PEGylation, site-selective modifications of proteins have been demonstrated with enzymatic transformation, i.e. protein prenylation, and chemospecific chemical modifications at the end terminus with pyridoxal 5'-phosphate [11]. Polymers mimicking the polysaccharide heparin when conjugated to basic fibroblast growth factor can preserve the bioactivity of this heparin-binding protein to stimulate proliferation of fibroblasts under a variety of stressors [12]. Zwitterionic polymers have also been introduced to conjugate proteins to increase their stabilities and it results in an improved binding affinity due to protein-substrate hydrophobic interactions [13]. Besides polymeric conjugation, techniques such as fusion protein chimeras with thermally sensitive peptides and non-covalent encapsulations of proteins into nanocomposites also preserve the activity of proteins [14,15••].

Approaches to control bioavailability

Retaining protein agents in hydrogel depots offers a locally concentrated reservoir for the long term delivery of therapeutics, ideally at a rate that coordinates with the pathological state of tissues.

Motivated by promoting long term availability of bioactive signals and therapeutics, direct tethering of peptide or protein molecules has been applied on engineered surfaces [16], three-dimensional hydrogel scaffolds [17] and nanoparticles [18]. Dynamic microenvironment of RGD adhesive peptides affects myogenic differentiation of myoblasts when photoactivatable peptides are functionalized on 2-D surfaces [16]. Tethering anti-inflammatory proteins on the surfaces of particulate nanocomposites prevents rapid clearance after intra-articular delivery to modulate osteoarthritis. An alternative to the direct tethering is to immobilize protein-binding molecules for target proteins to adhere via intrinsic affinities. Block copolymer-heparin conjugates were shown to complex into micelle structure for the retention and delivery of growth factors [19]. Single-stranded nucleic acids with high affinities to proteins, called aptamers, have also been incorporated into polymeric backbone to retain and prolong the release of proteins [20].

Thermally responsive conjugates on the proteins allow for the *in situ* formation of protein-depot composites upon temperature change, i.e. subcutaneous injection. For example, elastin-like peptides (ELPs) have been engineered to generate recombinantly fused protein-ELP chimera nanoparticles [21] and injectable depots [7]. ELPs are composed of a tandemly repeated sequence, (Val-Pro-Gly-X-Gly)_n, derived from the precursor and soluble form of elastin, which undergo a phase transition from an extended soluble structure to a collapsed insoluble one above its transition temperature. Elastin also has an intrinsic biological activity in inducing the proliferation of fibroblasts. As such, reserving growth factors in a smart injectable depot not only enables sustained release of protein therapeutics but also allows for seamless filling of tissue cavities.

Polymeric nano/micro spheres have been used in the development of a number of products in the market based on polylactide (PLA) and its copolymer with glycolide (PLGA). The clinical and commercial success of the first parenteral sustained-release formulation, Lupron Depot® utilizing PLA, propelled extensive studies and the resulted booming patents on the delivery of proteins using polymeric delivery systems. However, hydrolytically mediated extended release takes little consideration of the cellular microenvironment (i.e. pH or protease levels). In addition, the acidic environment from PLGA degradants and the hydrophobicity of the polymer may render proteins to deactivate. As such, other delivery systems have advanced to get around those limitations.

Liposomes are a widely used lipid bilayer platform to encapsulate drugs and vaccines with a number of clinically proven formulations. Assembled by the entropic driving force, this vesicle material raises concerns regarding its chemical and mechanical stability. A recent development by the Irvine group has covalently crosslinked the lipid headgroups across the opposing faces of adjacent bilayers with a pH-sensitive small compound, dithiolthreitol, within the vesicle walls, forming interbilayer-crosslinked multilamellar vesicles (ICMV's)

[22••]. The utility of these submicrometre-particle reagents was exemplified by entrapping protein antigens in the vesicle core and lipid-based immunostimulatory molecules in the vesicle wall, generating subunit vaccines which elicited strong T-cell and antibody response, i.e. ~1000 times and ~10 times greater humoral response than soluble antigen and non-crosslinked multilamellar vesicle immunizations in mice [23].

Another core-shell design to protect protein agents from environmental attacks and denaturations is to encase them in thin shell nanocapsules weaved with polymeric nano-sized matrices. Our laboratory has demonstrated the release of a single or multi-factorial proteins from these nanocapsules due to their degradable shell compositions that allow for the incorporation of a variety of environmental cues [15]. This platform has been successfully applied to encapsulate functional enzymes [24–29], growth factors [15] and even siRNA [30] with a number of advanced features, i.e. an enhanced and extended bioactivity, a specific cytoplasmic or extracellular delivery, a sustained release efficacy and flexible application routes of subcutaneous injection or embedded within scaffolds. Protein cargos can be either pre-modified or non-covalently entrapped within an *in situ* interfacial polymerized shell matrix. Different modalities have been included as release triggers to attain a cell-regulated long term delivery, such as intracellular or extracellular proteases, redox environments and photo-activations. Remarkably, release rates can be facilely tuned resulting in a differential delivery of intact cargos from these nano-reservoirs; and for the first time complementary protein complexes have been precisely assembled within these robust yet permeable thin shell matrices to greatly improve therapeutic efficiency, which opened up endless possibilities for the creation of novel molecular machineries.

Engineering multivalency or increased protein density

Nano-scale clustering of ligands can potentiate their binding abilities with receptors resulting in a signaling alteration and practical utilities, i.e. reducing dosage concentrations thus bringing down the cost. A biomolecular engineering strategy to present a precise valency of ligands is to fuse with bioactive molecules a component of the distinct coil-coil oligomerization sequences derived from different tissues, i.e. cartilage oligomeric matrix protein (COMP) for pentameric constructs [31]. Extracellular matrix molecule fibronectin contains the 7th to the 10th type III fragment (FNIII_{7–10}) for the recognition by integrin $\alpha_5\beta_1$. Covalent immobilizations of clustered FNIII_{7–10} on the surface of titanium implants showed an enhanced signaling efficiency by forcing integrin colocalizations and greatly improved the integration of the implants with surrounding tissues [32]. The recruitment of integrins can also synergize with the signaling of growth factor. The 12th to 14th fragment of fibronectin (FNIII_{12–14}) indiscriminately binds to several growth factors. A proximal co-presentation of FNIII_{7–10} and FNIII_{12–14} in the same polypeptide chain showed potent, synergistic effects in signaling and morphogenesis, but not when the two fragments were on different polypeptide chain [8]. As an alternative oligomerization strategy, dendritic polymers have been used to facilitate multivalent bindings resulting in an enhanced avidity between the ligand and the target [33]. Another strategy utilizes linear polymer for the multivalency of ligands, i.e. an angiogenic factor, Sonic hedgehog, or stem cell signaling ligand, ephrin-B2, which exhibited much more potency than the soluble form of a same concentration, exceeding the current standard approach of antibody-assisted clusterings

[34,35]. Lately our laboratory has designed nanoparticles comprised solely of heparin molecules to mediate the covalent immobilization of bioactive VEGF at different densities, which showed a potential in steering the migratory or proliferative pathways of endothelial cells in the angiogenesis process [36].

Delivery approaches

The therapeutic outcomes of peptide/protein agents are largely affected by routes of administrations. Systemic approaches offer a fast distribution of agents with minimal invasiveness, but may suffer from barrier issues. These difficulties can be in part solved by including modifiers in the protein formulations. Enhancers such as protein transduction domains or cell-penetrating peptides facilitate the cell permeation, whereas adjuvants such as chitosan or thiomers can lower the surface resistivity to promote paracellular transports. On the other end of the spectrum, local delivery has been applied in multiple situations with advantages such as minimizing non-target errors and creating a high local concentration and gradient. Research of both direct topical applications and surgical implantations has emerged actively to target various tissue types and organs. Growth factor nanocomposites are generally applied within hydrogel scaffolds comprised of natural extracellular matrices such as matrigel, collagen and fibrin, or synthetic mimics like alginate, PLGA/PLA, hyaluronic acid, self-assembling peptides. Exemplary current research of the local delivery of growth factors is summarized in Table 2.

Protein presentation impacts cell activation and tissue repair

As mentioned the ECM can bind growth factors with a high affinity resulting in their immobilization; this binding not only enhances the stability of growth factors but also modulates the fate and phenotype determination of nearby cells [1,2]. Elegant experiments temporally controlling the bioavailability and dose of vascular endothelial growth factor have demonstrated the delicate balance between appropriate dosing and time of release with therapeutic outcome in muscle as well as bone regenerations strategies [3,4]. In our laboratory we have demonstrated that the presentation of VEGF, bound, soluble, clustered or slowly released can each have different signaling responses to endothelial cells. For example, high density clusters of VEGF were developed using nanoparticles comprised solely of heparin [36]. High density VEGF clusters result in different responses in endothelial cell branching and VEGF-receptor 2 phosphorylation than less clustered and soluble VEGF. In addition, we have found that both covalently bound VEGF and electrostatically bound VEGF have differential signaling responses to endothelial cells [46,47] than soluble VEGF. In general, bound VEGF promoted migration (p38 activation), receptor clustering and integrin recruitment compared to soluble VEGF that promoted proliferation (p42/44 activation).

The spatial control of growth factors in 3-D hydrogel matrices presents tremendous chances in cell culture via its biomimetic gradient of concentrations and micrometer-resolved heterogeneity. Developed by the Shoichet group, agarose hydrogels with coumarin-caged thiols have been exploited for the creations of FGF-2 patterning [48], VEGF gradient [49•] and spatially controlled simultaneous multiple growth factor patterning in 3-D [50•].

Facilitated by multiphoton confocal laser, the intensity of the immobilized functionalities is correlated to the number of scans, while orthogonal physical binding pairs can be selected for mild immobilization conditions. The Chen and West groups utilized surface patterning to spatially control the presentation of VEGF in PEG hydrogel and observed accelerated endothelial tubulogenesis in micron-scale immobilized VEGF patterns [51]. It is worth noting that micro-patterning of the topology alone of hydrogel substrates, therefore restricting cell shapes, has shown to be necessary and sufficient to replace soluble factor signaling in mediating the commitment of stem cells to different lineages [52]. By solely manipulating the topology of hydrogel substrates, a gene-expression level towards myocardial lineage of mesenchymal stem cells was observed [53]. It remains to be investigated how these effects on gene expressions would ultimately impact the physical processes of tissue morphogenesis or regeneration. Lately, the tortuosity and geometry of microfluidic devices are also utilized to yield GF gradients that can modulate the morphogenesis of embedded cells [54]. To the authors' opinion, the *in vitro* replication of *in vivo* chemoattractant gradients is of particular interest because it provides an opportunity to affect signaling cascade and phenotypic changes of cells to simulate physiological environment.

The temporal control on the delivery of growth factors has traditionally comprised of tunable sustained dosages of singly factors and sequential releases of multiple factors. Other than chemical modifications or carrier developments discussed in the sessions of "Protein with a smart conjugate" and "Protein with a robust shell", sustained release strategies have heavily relied on affinity-based approach in the material design. Orthogonal physical binding pairs exploited for the tunable retention of growth factors in depots include biotin-streptavidin, barnase-barstar, Src homology 3 binding with proline-rich peptide [55]. In addition, the binding domains for various types of growth factors have been found on extracellular matrix molecules such as heparin, collagen, fibronectin and vitronectin. The simultaneous or sequential delivery of multiple GFs for aiding tissue constructs should be selected based on the synergistic and combinatorial effects of the proteins of interest. For example VEGF and Ang-2 are pro-angiogenesis factor that cooperatively promote endothelial sprouting and pericyte detachment from pre-existing vessels, whereas pro-maturation factors PDGF and Ang-1 when added subsequently following VEGF/Ang-2 promote vessel maturation and vascular remodeling [56•].

Other protein nanomaterials

While studies of appropriate vehicles have blossomed with novel designs mentioned above and clinically proven synthetic materials, research in untapped areas of protein delivery have shown exciting advances. Examples as facilitating the formation of extremely high concentrations of proteins, building protein "lego" molecular machines and reconstructing artificial cellular organelles are emerging as new alternatives (summarized in Table 3).

Conclusions

As more and more technologies have been realized to improve the nanocomposition, microenvironment and delivery vehicles of protein agents, new discoveries of the biological

nature of cells, bioactive cues and extracellular matrices continue to offer insights into the design and engineering of materials and biologics. For example, the immobilization of secreted proteins with extracellular polysaccharides inspired designs of polymers to offer multiple features, i.e. stabilizing therapeutic proteins and providing desirable pharmacokinetic behaviors. Meanwhile, immobilized proteins can be changed in their binding affinity to the cognate receptors, offering new possibilities of engineering ligand-receptor interaction. While smart delivery of proteins can successfully target the pathological sites by incorporating different environmental cues, the mode of presentations, i.e. release profiles and multivalency, and its immediate biological neighborhood such as synergistic partners, strongly affect the therapeutic efficacy of proteins. Therefore in the design of protein nanocomposites, multiple factors should be carefully considered including but not limited to protein stability, bioavailability, multivalency, delivery, and presentation. Moreover, incorporating cellular therapeutics, i.e. stem cell therapy or transgenic cells, in combination with protein agents may lead to a most beneficial outcome in tissue repair and regeneration.

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Highlights

- Biomimetic polymers and peptides stabilize protein structure for long-term activity.
- Clever nanodepots protect and cell-regulatedly release GFs in a slow sustained way.
- Multivalent/cluster presentation of GFs changes the dynamics of receptor activation.
- Fibronectin fragment fusions synergize integrin with receptor activation by GFs.
- Orthogonal binding pairs assist protein binding and spatial control in hydrogels.

Figure 1.

Recent advances in nanotechnology to address the leading challenges in the applications of protein-based therapeutics. Segments are divided according to the order of appearances of the corresponding paragraphs in this paper and are clockwise oriented in this figure starting from the upright position. See text for detailed description and references.

Table 1

Representative clinical trials for GF-based therapies for tissue repair Source: <http://www.clinicaltrials.gov>

Condition	Application	GF type	GF carrier	Route	Phase	Sponsor
Periodontitis (inflammation of tooth bone or ligaments)	Alveolar bone increase rate	bFGF ^{a,2}	3% solution of hydroxypropylcellulose	Injection during flap operation	3	Kakan
Melanoma(IV)	Suppressing plasma FGF	Interferon alpha-2b	PEG ^b -ylated	weekly SC ^c	2	Eastern Cooperative Oncology Group
Heart failure (ischemic cardiomyopathy)	Autologous cardiac-derived stem cell	bFGF controlled release	Gelatin hydrogel sheet	Intra-myocardial injection	1	Naofumi Takehara
		Ad-HGF ^d		Trans-endocardial injection	1/2	Jiangsu Province Hospital
		IGF ^e -1 (mecasermin)	0.9 % NaCl solution	Intracoronary injection	1/2	University College Cork
		hVEGF ^f -165	Mini-thoracotomy	intramyocardial injection	2	Instituto de Cardiologia do Rio Grande do Sul
Neuropathic diabetic foot ulcer	Wound closure	bFGF (Trafermin)	Spray	Topical	3	Olympus Biotech
		EGF ^g			3	Daewoong Pharmaceutical
Venous leg ulcer		5-amino acid deleted rhHGF (CHRONSEAL ®)		Topical	1/2	Kringle Pharma
Burn wound		EGF	Silver sulfadiazine cream	Topical	2/3	Chulalongkorn University
Cartilage injury repair	Complete healing of 3 rd degree thermal electrical burn	rhPDGF ^h (R-Pdf/Gbb)	0.01% gel	Topical	2	American Scitech International, Johnson & Johnson
	knee	Human FGF18 (AS902330)		Weekly intra-articular injection	2	Merck KGaA
lateral epicondylitis (soreness or pain on the outside of the upper arm near the elbow)	Tennis elbow	rhPDGF	sodium acetate buffer	injection	2	BioMimetic Therapeutics
Tendon injury	Rotator cuff	rhPDGF	Fibrin matrix	Local during surgery	3	Hospital for Special Surgery, New York
Skin lesion	Rash side effect from Erlotinib	EGF	ointment	Topical	2	Dong-A University Hospital
Oral Mucositis	Preventive during chemotherapy	EGF	With povidone iodine, chlorhexidine, & nystatin	Topical twice daily	2	Seoul National University Hospital
Fulminant hepatic failure	Unable to receive liver transplantation	rhHGF			1/2	Kyoto University
Hepatitis C-related cirrhosis	Hepatocellular Carcinoma Prevention	Interferon alfa-2b	pegylated		3	Schering-Plough

Condition	Application	GF type	GF carrier	Route	Phase	Sponsor
22q13 Deletion Syndrome; RETT Syndrome	Children	IGF-1		SC injection	2	Mount Sinai School of Medicine; Children's Hospital Boston
Idiopathic Short Stature		r-hGF (Saizen®)		SC	3	Merck KGaA
Prevent autoimmunity	Promote thymic T cell	KGF ⁱ [Palifermin (Kepivance®)]	Bolus	IV/ injection	1/2	Cambridge University Hospitals NHS Foundation Trust
Lung	Airway endothelium; Asthma treatment	KGF	Bolus "collapsed"	IV injection	2	University Hospital Southampton NHS Foundation Trust
Brain	Improve neurological function	NGF ^k		Intranasal	2	Jinling Hospital, China
Eye	Neurotrophic keratopathy	KGF		Eye drop	1/2	Dompé s.p.a.
Stroke	Preventive	E-selectin		Nasal	1	NINDS ^l
Bone	Stimulate ossification	BMP ^{m-2}	Collagen type 1	Local injection	1	Northern Orthopaedic Division, Denmark

Abbreviations:

^a fibroblast growth factor,

^b polyethylene glycol,

^c subcutaneous,

^d hepatocyte growth factor,

^e insulin-like growth factor,

^f vascular endothelial growth factor,

^g epidermal growth factor,

^h platelet-derived growth factor,

ⁱ keratinocyte growth factor,

^j intravenous injection,

^k nerve growth factor,

^l national institute of neurological disorders and stroke,

^m bone morphogenetic protein.

Table 2

Growth factors in hydrogel-based therapy for tissue repair

Tissue	Animal model	Details	Refs
Bone	8mm calvarial defects in rats	- Dual delivery of rhPDGF-BB^a (transient) and BMP2^b-expressing BMSCs^c (long term) in collagen gel - Suppressive modulation of PDGF-BB on osteogenic differentiation and receptor expression of BMSCs - Enhanced bone formation & mineral density in vivo	[37]
	8mm mid-femoral defect in rats	BMP2-containing alginate gel within nanofiber mesh tube, in comparison to collagen sponge	[38]
Brain	Stroke in mice	Epi-cortical delivery of EPO^d, PEGylated EGF^e or in PLGA^f particles in hyaluronic-methylcellulose gel to stimulate endogenous NSPC^g and promote tissue repair	[39–41]
Heart	Cardiac ischemia-reperfusion in rats	- PLGA microparticles for sustained VEGF^h delivery - After 1 month, increase in angiogenesis and arteriogenesis and greater LVⁱ wall thickness	[42]
Skin	Mouse deep burn-wound model	Engineered human vasculature in hyaluronic acid hydrogel implanted to cover wound for integration with host vasculature and regression later during remodeling	[43]
Spinal cord	Subacute spinal cord injury in rats	- NT-3^j, PDGF in fibrin scaffold delivering mouse embryoid bodies of ESNPCs^k - Inclusion of heparin did not make a difference in increasing number of ESNPCs but ESNPC-derived NeuN^l-positive neurons	[44]
Other	Bladder lesion 2.2mm in diameter in rats	IGF-1^m fused with a substrate sequence tag derived from α_2PI₁₋₈ⁿ is covalently incorporated in fibrin for sustained release at lesion site stimulated considerable smooth muscle cells and host tissue response	[45]

Abbreviations:

^a recombinant human platelet derived growth factor (BB homodimer),^b bone morphogenetic factor 2,^c bone marrow mesenchymal stromal cells,^d erythropoietin,^e epidermal growth factor,^f poly(lactic-co-glycolic acid),^g neural stem/progenitor cell,^h vascular endothelial growth factor,ⁱ left ventricle,^j neurotrophin 3,

k embryonic stem cell-derived neural progenitor cell,

l neuronal nuclei,

m insulin-like growth factor 1,

n α 2-plasmin inhibitor

Table 3

Non-conventional nanocomposite designs and delivery approaches

Considerations	Type	Details	Refs
High loading concentration	Cosolutes	- Introducing trehalose to induce nanoclustering of naked proteins (up to 400 mg/mL) in dispersion - Viscosity is well below SC injection limit and shows normal pharmacokinetics	[57,58]
Protein "lego"	Self-assembly	12-subunit 16-nm cage assemblies was obtained with atomic level accuracy by designing the two oligo building blocks and their interface linking component	[59,60]
Protein-producing device	Cell-free system	Transcriptional and translational components are put in an artificial boundary for the photoactivated synthesis of proteins	[61]
Carrier, vector-free	Shear-induced	- Compression and shear forces result in transient holes in cell membranes for diffusion delivery of diverse biomolecules into cell cytosol - High throughput rate and cell-type independent	[62]
Enzyme-assisted photolithography	MEMS ^a	Micron-scale patterning of peptide ligands or antigen for cell patterning and sorting	[63]
Source-host separation	Microfluidic	Parallel channels hosting GF cocktails and endothelial cell covered artificial vessels to study angiogenic sprouting morphogenesis	[64**]
Direct cellular uptake & good serum stability	Lipid-core nanoparticle	Supramolecular self-assembled peptide-modified Au-NP stabilizes proteins on the exterior of a lipid-core capsule	[65]

Abbreviation:

^a micro-electro-mechanical system.