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Nitric-Oxide-Releasing Biomaterial Regulation of the Stem Cell Microenvironment in Regenerative Medicine

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Stem cell therapy has proven to be an attractive solution for the treatment of degenerative diseases or injury. However, poor cell engraftment and survival within injured tissues limits the successful use of stem cell therapy within the clinical setting. Nitric oxide (NO) is an important signaling molecule involved in various physiological processes. Emerging evidence supports NO's diverse roles in modulating stem cell behavior, including survival, migration, differentiation, and paracrine secretion of proregenerative factors. Thus, there has been a shift in research focus to concentrate efforts on the delivery of therapeutic concentration ranges of NO to the target tissue sites. Combinatory therapies utilizing biomaterials that control NO generation and support stem cell delivery can be holistic and synergistic approaches to significantly improve tissue regeneration. Here, the focus is on recent developments of various therapeutic platforms, engineered to both transport NO and to enhance stem-cell-mediated regeneration of damaged tissues. New and emerging revelations of how the stem cell microenvironment can be regulated by NO-releasing biomaterials are also highlighted.

1. Introduction

Combinatory therapy using biomaterials in conjunction with stem cells and the controlled release of protective prosurvival factors, such as the gasotransmitter nitric oxide (NO), are effective and holistic approaches to significantly improve tissue regeneration, by overcoming the limitations of each individual component. Biomaterials provide adherence and stability to transplanted stem cells,[1] and can regulate the otherwise rapid release of NO.[2] Recent revelations have shown how in response to local NO release, stem cells can secrete proregenerative and immunoregulatory factors to provide a supportive tissue microenvironment for tissue repair.[3]

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Since it was first reported within the cardiovascular system, the potent free-radical NO has been a major point of research within biological and clinical sciences.[2] NO was thereafter named "Molecule of the Year" in 1992 by the journal *Science*, and received the 1998 Nobel Prize for Physiology and Medicine.[4] NO has a broad range of physiological actions within the cardiovascular, musculoskeletal, nervous, and immune systems.[5] NO is endogenously synthesized by NO synthases (NOS), endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), or by conversion from nitrite ions. NO-based therapeutics have become a popular focus for the treatment of cancers due to potent antitumor effects,[6] and features prominently in therapeutic strategies for cardiovascular and neural diseases.[7] More recently the diverse effects of NO administration on progenitor and stem cells have become apparent,[3] opening new avenues for potentiating stem cell and biomaterial-based therapies in regenerative medicine.

NO activity on stem cell behavior does appear to be tissue-specific, but it is clear that NO can exert cell protective or apoptotic effects (low concentrations, nmol) and cell cycle arresting effects (high concentrations, nmol–μmol); this diverse range of activities may signify the reason there are few FDA-approved and marketable treatments. NO reacts with superoxide radicals (OH, O_2), forming oxidants peroxynitrite (ONOO), nitrite (NO_2^-), and nitrate (NO_3^-), which can result in NO inactivation and intrinsic deficiency, contributing to DNA damage and lipid peroxidation,[8] and pathological conditions including atherosclerosis, inflammation, dysfunctional wound healing, and tumor development.[9] In addition, the reactive nature of NO and its short half-life limits its sphere of influence to a few micrometers, restricting NO efficacy from reaching target sites.[10] Hence, the controlled release of NO by biomaterials has emerged as popular aspects of regenerative medicine research.

In this progress report, we discuss NO-releasing biomaterials, with a specific emphasis on their influence on stem cell behavior, function, and alteration of the cell microenvironment, as related to regenerative applications. Biomaterial platforms have emerged as promising approaches to overcome challenges associated with biological administration and promotion of spatiotemporal generation of physiologically relevant concentrations of NO. We begin with discussing progress in recent advances in NO donors, then understanding NO influence on stem cells and the stem cell microenvironment, their

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incorporation into biomaterials, and finally conclusions and outlook for NO-stem cell co-therapy.

2. NO Donors and Regulation of Stem Cells

2.1. Trends in Controlled NO Release by NO Donors

NO donors are pharmacologically active carriers of NO, which can assist in the delivery of NO to target tissues for localized release. Factors such as enzymatic cleavage, pH alterations, temperature, light, and reactions with metals or thiols can further promote the release of NO from donor molecules.[11] Thus, NO donors can be tailored to exhibit a wide range of NO release profiles. Of the four main types of low-molecular-weight (LMW) NO donors: *N*-diazeniumdiolates (NONOates), *S*-nitrosothiols (RSNOs/SNOs), metal–nitrosyl complexes, and nitrates. The most commonly investigated LMW NO donor type, NONOates, are synthesized by reaction of gaseous NO with secondary amine groups, under high (5 atm) pressure conditions.[2] At physiological conditions, NONOates spontaneously decompose to release two moles of NO per mole of donor. However, cationic primary amines can be used to electrostatically stabilize the anionic diazeniumdiolate groups, giving rise to a multitude of NONOates with broad ranges of release half-life (2 s to 20 h).[12] Synthesized NONOates include diethylamine (DEA), diethylenetriamine (DETA), dipropylenetriamine, and proline NONOates. The well-described characterization, ease of modification, protonation-responsive release mechanism, and relatively high thermal stability make NONOates the most attractive choice for conjugation onto components for biomaterial fabrication.

RSNOs act as physiological transporters of NO in vivo.[13] RSNOs can be chemically prepared by reaction of NO_2, N_2O_4, N_2O_3, or $NO_2\cdot$ with thiol-containing materials, such as alkyl nitrite and nitrous acid. The release kinetics of RSNOs is dependent on temperature, light irradiation, and the presence of metal ions.[14] Two stable RSNO compounds most commonly used in experimental studies are SNAP and *S*-nitrosoglutathione (GSNO).[15] More recently, increasing attention has been given to the development of new classes of donors with enzyme responsive release, reactive oxygen species (ROS) responsive release, and light-inducible NO release. The latter of which, are typically based on metal–NO complexes and nitrobenzenes,[14c] giving rise to a plethora of new NO donor strategies, effectively reducing unspecific NO release. The majority of recently developed NO donors have been applied in anticancer settings,[16] followed by cardiovascular and antimicrobial applications.[2,17] For a more comprehensive overview of the numerous NO donors and recently developed donors for a wider range of applications, we refer the reader to the review by Oliveira et al.[11]

The precise on-demand release of NO is a common objective shared by multiple research teams around the world. The rationale for the shift into the development of "smart donors" that respond to environmental factors rather than spontaneous release is clear; accuracy in the localization of NO release is required for tissue- and cell-specific responses in regenerative medicine applications. Biological systems use enzymes to regulate levels of endogenous NO. Inspired by such principles in nature, synthetic enzyme-responsive donors have been reported. These offer the advantages of supplying highly localized, stable, and continuous NO, adjustable by administration of the corresponding enzyme and thus improving pharmacokinetics NO release. While an abundant range have been developed, enzyme-responsive donors susceptible to NO biocatalysis by glycosidases such as β-galactosidase,[18–20] and glutathione S-transferases[21] are the most commonly used and have formed the basis of a new generation of enzyme prodrug–based therapies. ROS-sensitive donors offer advantages for the induction of NO release under pathological conditions, such as tissue hypoxia. An intuitive donor was recently designed by Hou et al.,[22] wherein a mitochondria-targeted superoxide-responsive NO donor (MitoSNOD) was synthesized to include three specialized moieties: superoxide-responsive diphenylphosphinyl, NONOate, and mitochondrial-targeting triphenylphosphonium. Upon contact with superoxide, diphenylphosphinyl underwent a self-immolative cascade reaction to release NO. Thus, MitoSNOD exhibited dual functionality: the specific release of therapeutic NO in proximity to mitochondria, while scavenging ROS generated. Light-responsive donors respond directly to external light stimuli, as a result heat or ROS are generated to trigger NO release, permitting a specified region of release with tunability correlated to the light source used. Recently, laser light or near infrared (NIR) light induction

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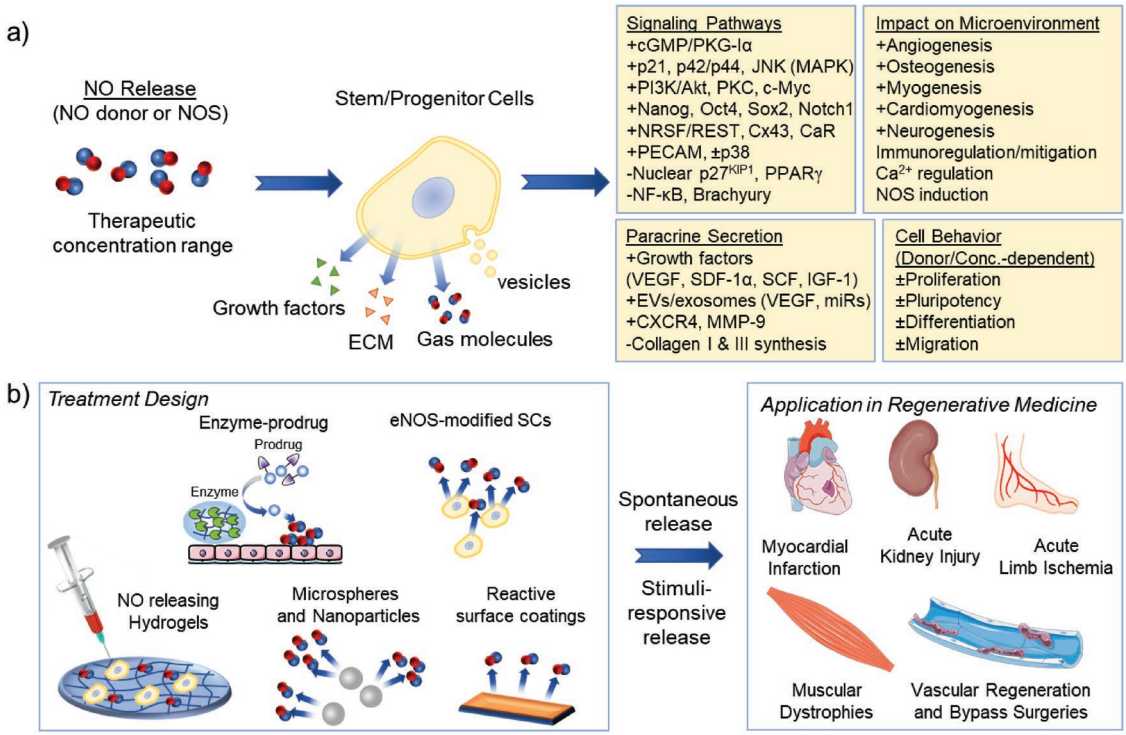

Figure 1. a) Summary of the effect of NO release on stem cell signaling pathways, paracrine secretion, behavior, and impact on the surrounding microenvironment. b) Summarized treatment designs for the incorporation of NO release and stem cell co-delivery into biomaterials and potential applications in regenerative medicine.

of release have become preferential as these methods of emission can penetrate deep tissues with limited adverse effects. Guo et al.[23] developed SNO-containing NO nanogenerators that absorbed NIR laser light, generated heat via photothermal conversion, and subsequently promoted the breakdown of S—NO bonds for NO release. Blangetti et al.[24] created an organic NO photoreleaser, (Z)-1, which incorporates the spontaneous NO donor Cupferron, masked by a boron-dipyrromethene (BODIPY)-derivative. Photoexcitation of the BODIPY with a single green light photon induced heterolytic cleavage and unmasked Cupferron, resulting in subsequent NO release. As such, enzyme-, ROS-, and light-responsive NO donors are the optimal donors for further development and incorporation into biomaterials. As new physiochemical parameters and modes of release from these smart donors are developed, incorporation into biomaterials can help to facilitate maximal performance. Potential NO donor-biomaterial combinations are staggering. Therefore, this progress report will selectively evaluate what are, in our opinion, the most promising platforms for conjunction with stem-cell-based therapies.

2.2. The Effects of NO on Stem Cell Function

NO treatment has been reported to result in changes in stem cell function, including paracrine secretion pattern, release of growth factors and extracellular vesicles/exosomes (**Figure 1**A). These factors influence how stem cells interact with other cells and the tissue microenvironment, implicating the design of biomaterial-based treatments as promising regenerative medicine approaches to clinical problems (Figure 1B). Information from this early area of research remains limited. Below, we summarize the known effects of NO release on stem cell secretion and the impact on the microenvironment, and the implications of these in utilizing biomaterials for regenerative medicine.

2.2.1. Growth Factor Release

Endogenous NO was documented to drive stem cell factor (SCF) release by neural stem cells (NSCs) in embryonic neurons.[25] Chen et al.[26] demonstrated that NO co-treatment alongside tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) significantly induced insulin-like growth factor 1 (IGF1) secretion by radiation-induced inflammatory-state bone-marrow-derived mesenchymal stem cells (BMSCs), improving intestinal injury. Overall, the full extent of growth factor release by stem cells remains unknown.

2.2.2. Induction of Paracrine Signaling and Transcription

Besides the well-documented activation of PI3K/Akt and cGMP-PKG pathways by NO,[27] in NSC cell cycle progression, NO initiated p21Ras/MAPK-dependent decreases in nuclear presence of cyclin-dependent kinase inhibitor 1 ($p27^{KIP1}$), surprisingly independent of epidermal growth factor (EGF) receptor

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activation, which was originally thought to be the principal upstream receptor.[28] Excessive NO exposure promoted transition of neural progenitor cells (NPCs) into glial cells, dependent on transcription factors neuron-restrictive silencing factor and repressor element silencing transcription (REST) activity, transforming the consensus that neural signaling was reliant on high levels of NO exposure alone.[29] NO activation of c-Jun N-terminal kinase (JNK)/MAPK signaling promoted osteogenic differentiation, while reducing adipogenic differentiation of periodontal ligament stem cells (PDLSCs), dependent on.[30] Arguably, the most comprehensive study on stem cell pathway activation by NO was performed by Nogueira-Pedro et al.,[31] wherein hematopoietic stem cells (HSCs) incubated with NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) resulted in a short-term period of activation of a range of signaling proteins. This brief window of activated signaling events was deemed imperative for HSC differentiation into granulocyte–macrophage precursors. When taken together with NO regulation of pluripotency-associated transcription factors (Nanog, Oct4, Sox2, Brachyury),[32–34] it is evident that NO operates through precise temporal activation of intracellular signaling pathways and transcription factor activation of gene expression, imperative in regulating stem and progenitor cell function or fate. Another interesting paracrine effect was described by Wang et al.,[35] wherein NO-inhibited human embryonic stem cell (hESC) derived cardiomyocyte expression of calcium release-activated calcium channel protein 1 (Orai1) and store-operated Ca^{2+} entry (SOCE), attenuating cardiac hypertrophy and protecting cells from harmful Ca^{2+} overload,[36] implicating the use of stem cells with NO-releasing and cardiomyocyte-promoting biomaterials in cardiovascular diseases.

2.2.3. Immunoregulation

Recent studies have drawn increasing attention to stem cell mitigation of the immune response by NO production, implicating NO administration at physiological levels in achieving immunoregulation during pathological conditions. NO production was suggested to act as a prominent molecular switch in MSC-mediated immunomodulation, either promoting or attenuating T-cell proliferation.[37] It has become apparent that immune suppression by mesechymal stem cells (MSCs) is dependent on combinational release of NO with proinflammatory cytokines, such as interferon-γ (INF-γ) and TNF-α.[38] MSCs have been shown to suppress the excessive immune responses of B-cells, T-cells, macrophages, natural killer cells and dendritic cells via combined activities of numerous immunosuppressive mediators, inducible by NO. Therefore, we speculate that combinational treatment by NO-releasing biomaterials and stem cells would serve to vastly improve efficacy of treatments of inflammatory diseases and graft survival of materials susceptible to foreign body immune rejection. Complete mechanisms of NO-dependent MSC regulation of immune cells have yet to be elucidated; for a more in-depth coverage of MSC immunoregulation and regulation of T-cell function, we refer the readers to reviews by Shi et al.[39] and Duffy et al.,[40] respectively.

2.2.4. Regulation of Stem or Progenitor Cell Stemness

Several studies have demonstrated the influence that NO administration can have on progenitor and stem cells (**Table 1**). Over the past 5 years, increasing scientific understanding of NO regulation of various types of progenitor and stem cells has given insight into how NO and stem cell co-therapy can provide distinct benefits for tissue repair. Recent investigations have sought to establish NO regulation of progenitor and stem cell "stemness," primarily within the context of disease progression and tissue development. For the most part, these studies have utilized NO donors of varying types and concentrations in conjunction with various types of stem or progenitor cells. However, aside from a low range of NO donor concentrations (2–50 μM), a firm consensus based on exact NO-donating material parameters to achieve a consistent effect, has yet to be determined.

Table 1. NO-donor and depletion-based treatments and influence on stem cell behavior.

Treatment type	Stem/progenitor cell	Effect on stem cell behavior	Ref.
NO donors			
SNAP	ESCs	Enhanced cardiomyogenesis	[41,42]
		Enhanced osteogenesis	[43]
	ESC-derived cardiomyocytes	Inhibited ESC-cardiomyocyte hypertrophy	[35]
DEA	ESCs	Enhanced cardiomyogenesis	[41]
PAPA	ESCs	Enhanced cardiomyogenesis	[41]
NOC-18	ESCs	Enhanced cardiomyogenesis	[42]
	DPSCs	Promoted odontogenic lineages	[44]
DETA	ESCs	Prevented apoptosis from LIF-depletion	[34b]
		Induced mesodermic lineages (myogenesis, vasculogenesis)	[34a]
	MVSCs	Inhibited proliferation and SMC differentiation	[9a]
	mESCs	Preserved pluripotency, reduced proliferation	[33]
Molsidomine	FAPCs	Prevented fibro-adipogenic lineages	[45]
	eVE-Cad^+ EPs	Enhanced embryonic myogenesis	[5b]
NOD560	MSCs	Attenuated migration	[46]
NO depletion			
GSNO reductase	MSCs	Promoted vasculogenesis	[47]
L-NAME	ESCs	Promoted EC differentiation (unstable phenotype)	[48]
		Inhibited cardiomyocyte differentiation	[42]

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The NO donor molsidomine used in vivo at 3 mg kg^{-1} significantly reduced differentiated mesenchymal fibro-adipogenic progenitor cells (FAPs) within tibialis anterior muscles of *mdx* mice, suppressed fibro-adipogenic cellular function, reduced collagen expression and inhibited adipogenesis.[45] Whereas the same donor used at the same concentration promoted differentiation of skeletal muscle-associated embryonic VE-Cadherin-expressing progenitors (eVE-Cad^+) into self-renewing mesoangioblasts (MABs).[5b] These contrasting studies highlight the important ramifications of NO effect on pathophysiology of adult muscular dystrophies, as well as on early embryogenesis, myogenesis, and related signaling pathways. Thus, the source of stem cell used, and the desired regenerative application must be carefully considered in the design of NO-releasing biomaterials for stem cell-based therapies. Sonoda et al.[44] demonstrated NO-induced odontoblast-specific differentiation of dental pulp stem cell (DPSC) populations by the NO donor 1-hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazen (NOC-18), resulting in enhanced mineralized tissue formation. Leukemia inhibitory factor (LIF) promotes stemness by inducing pluripotency-associated transcription factors Nanog, Oct4, and Sox2 while suppressing Brachyury.[32] As little at 2 μM of NO donor DETA could achieve a similar effect to LIF, effectively locking mouse embryonic stem cells (mESCs) into a pluripotent, non-proliferative state, and significantly delaying differentiation.[33] Other stem cell function can also be regulated; He et al.[46] used *N*-nitroso rhodamine (NOD-560), an NO donor with photoinducible NO release to show on-demand inhibition of a MSC migration, although the exact mechanism of action was not detailed.

3. NO-Releasing Biomaterials and Application in Stem Cell Therapy

Aside from the pleiotropic effects of NO on stem cell pluripotency or differentiation, modulation of the stem cell secretome is evident. As further revelations emerge, researchers must consider how to best fine-tune materials to exploit stem cell impact on the tissue microenvironment and maximize therapeutic efficacy. NO donor drugs are useful tools for determining NO effect on cellular functionality, however their disadvantages limit their further applications in vivo, and these include cytotoxicity, thermal/photochemical instability, limited NO payload, and lack of specificity for targeted and controlled NO release. Thus, there has been enormous effort in seeking biomaterial-based strategies to load NO donors and deliver precise amounts of NO directly to the target tissue site, in a controlled manner, with minimum adverse effects.

Functional NO-releasing platforms have been utilized to administer NO to target tissue sites, in a controlled manner over the course of the envisaged treatment.[2] However, the combinational use of biomaterials, NO generation and stem cells is still a relatively new field in regenerative medicine (**Table 2**), and continues to develop as the roles of controlled NO release and influence on stem cells and their microenvironment are better understood. Below, we focus on recent (within the previous 5 years) applications of biomaterial platforms for sustained delivery of NO and their implications in stem cell therapies. We have predominantly reviewed materials that have been utilized for stem cell therapies, we also discuss related materials that could have facile translation potential for integration with stem cell therapies. For each of the biomaterial classes highlighted, we address the choice of donor, compatibility with the material, their conjugation chemistry and NO release mechanisms.

3.1. Biomaterials

3.1.1. Hydrogels

A major challenge in stem cell therapy is the design and fabrication of scaffolds that mimic native extracellular matrix (ECM), support cell survival and orchestrate stem cell repair of functional tissues. Hydrogels are hydrophilic and biocompatible materials with relatively easy tunability for recreating ECM properties. Besides these properties, hydrogel delivery systems are useful tools for maintenance of high local concentration of therapeutic agents released at specific sites. Hydrogels have flexibility in their use, they can be directly attached onto pathological sites of tissues or they can be deposited on medical devices such as implants or stents. There exists abundant methodology for the synthesis of hydrogels incorporating effective means of sustained NO release.

In our recent studies, we employed enzymatic dictated release in combination with biodegradable polymer hydrogels, to provide sustained and gradual NO release. We used galactose caged NO donors (Gal-NONOate) and through click chemistry, synthesized chitosan-NO (CS-NO) injectable gels, cast membranes, and porous scaffolds.[58] On-demand release of NO was achieved by blocking the decomposition process of the modified CS-NO by galactose, only after exposure to and glycosidic bond hydrolysis by β-galactosidase could NONOate become unprotected and release NO. Controlled NO release by CS-NO increased the angiogenic and ischemic therapeutic potential of hP-MSCs (**Figure 2**).[49] We also investigated the capacity for hydrogels formed from CS-NO to promote endothelial cell (EC) lineage commitment from ESCs.[27b] We used CS-NO hydrogels as cell-free feeder systems for ESCs, resulting in selectively up-regulated EC markers (VEGF-receptor 2 and VE-Cad) and EC differentiation through PI3K/Akt-pathway activation. Controlled NO release drove EC lineages from stem cells without the addition of expensive growth factor cocktails, thus providing a feasible in vitro solution for EC-based therapies in vascular repair.

We recently transplanted adipose derived-MSCs (ADSCs) within a naphthalene-peptide hydrogelator with β-galactose caged NONOate (NapFF-NO).[19] The β-galactose caged NO donor was covalently conjugated to the short FFGGG peptides by Cu^I catalyzed click reaction of azide and alkyne side chains, respectively. ADSCs were co-transplanted with NapFF-NO into murine myocardial infarction (MI) models and administration of β-galactosidase by tail vein injection at the time of surgery, demonstrated improved cell survival within the engraftment site, over 7 days. Besides enriching the microenvironment with vascular endothelial growth factor (VEGF)

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Table 2. NO-releasing biomaterial-based treatments and influence on stem cell behavior.

Treatment type	Stem/progenitor cell	Effect on stem cell behavior	Material-related strategy	Ref.
CS-NO	hP-MSCs	Promoted angiogenesis	β-Gal caged NONOate donor conjugated to biocompatible CS polymer string backbone for ischemic injury; β-galactosidase enzyme-responsive release	[49]
PPC–nanofiber graft	eNOS-modified BMSCs	Sustained eNOS and NO production	Genetically modified BMSCs overexpressing eNOS seeded onto electrospun non-woven microfibers of poly(propylene carbonate) vascular grafts; genetically modified BMSCs as source of NO	[50]
SNAP–gelatin hydrogel	MSCs	Attenuated attachment and proliferation	Prohealing gelatin hydrogel; spontaneous release of NO from SNAP conjugate	[51]
eNOS-pDNA hydrogel	ADSCs	Synergistic increase in cardiomyogenesis	Combined ADSC and eNOS-pDNA nanocomplexes within injectable in situ forming TA-PEG/HA-SH hydrogel; NO generation reliant of eNOS-pDNA transfection	[52]
NapFF-NO hydrogel	ADSCs	Enhanced cardiomyogenesis, survival	β-Gal caged NONOate donor conjugated to NapFF hydrogelator for ischemic injury and ESC survival; β-galactosidase enzyme-responsive release	[19]
CS-NO hydrogel	ESCs	Promoted EC differentiation	β-Gal caged NONOate donor conjugated to biocompatible CS hydrogelator; β-galactosidase enzyme-responsive release	[27b]
Peptide-NO nanomatrix	EPCs	Promoted EC differentiation	Self-assembling peptide nanomatrix surface coatings for ischemic injury; MMP2-mediated release of peptide-caged polylysine–NO donor	[53]
SNAP-ECM scaffold	MSCs	Inhibited MSC activity, altered morphology	Biocompatible and structural ECM-like scaffold for wound healing; spontaneous release of NO from SNAP conjugate	[54]
dNat vascular tissue	eNOS-modified ADSCs	Promoted EC differentiation	Genetically modified ADSCs to overexpress eNOS, seeded onto decellularized native vascular tissue grafts; ADSCs as the source for NO release	[55]
PEI/NONOate-PLGA microspheres	GMSCs	Increased proliferation, osteogenic differentiation	GMSC/NO-donor heterospheroid microspheres for administration to periodontal defects; spontaneous release of NO from NONOate	[56]
NIR-UCNPs	CSCs	Induced apoptosis	Nanoparticles for highly efficient uptake by cancer stem cells; NIR light-triggered breakdown of RBS, releasing coupled NONOate	[57]

and stromal-cell-derived factor-1α (SDF-1α), treatment provoked endothelial cell (EC) migration, and ameliorated heart function. Very recently, Ou and Yang et al. synthesized a similar bifunctional and self-assembling supramolecular and nanofibrous hydrogel that formed within 1 h in the presence of glutathione, after mixing a 4:1 ratio of curcumin/peptide derivative (Cur-FFE-ssERGD) and NapFF-NO.[59] This mixed component hydrogel was stable for up to 1 month postsynthesis. Release kinetics demonstrated sustainable and low concentration release of the antioxidant curcumin by ester bond hydrolysis. Moreover, NO release could be controlled over a period of at least 48 h upon β-galactosidase administration and catalysis of the glycosidic bond protecting NONOate from spontaneous release. In vivo studies showed that this hydrogel could efficiently reduce ischemic reperfusion injury and myocardial cell autophagy/apoptosis by inhibiting the ROS-associated p38/NF-κB pathway, as evaluated 30 days after surgery.

The use of smart donors in hydrogels circumnavigates the associated issues of burst release. SNAP conjugated to gelatin hydrogels showed rapid NO release (for 2 h) followed by an impressive sustained release (for 70 h).[51] However, the outcome was reduced MSC attachment, proliferation, and rounded MSC morphology. Cell-derived ECM scaffolds incorporating SNAP (SNAP-ECM) were fabricated to release NO to mimic in vivo NO release kinetics within the stromal environment during wound healing.[54] This too resulted in impaired MSC cell growth, altered morphology, disrupted cytoskeletal F-actin organization and focal adhesion-related molecules. These results may demonstrate the detrimental effects of micromolar NO concentrations on MSCs within the wound environment but may also be indicative of how macrophages regulate and organize stromal cell activity through NO generation during the inflammation stage of wound healing. We suggest that the outcomes of healing could be improved with additional restriction through caged donors, lowering the range and rate of NO release, as in our previous study.[60]

The drawback of NO donor-hydrogels is the limited pool of NO available. For short-term treatment, this may be ideal, but for long-term treatment, the effects of NO are difficult to sustain. Wang et al.[52] opted for an alternative approach to using NO donors that has the potential to overcome restricted payload and depletion of NO supplies in hydrogels. The research team developed an injectable conductive hydrogel comprised of tetraaniline-PEG diacrylate (TA-PEG) and thiolated hyaluronic acid (HA-SH). The hydrogel formed in situ upon injection and was mixed with nanocomplexes containing plasmid

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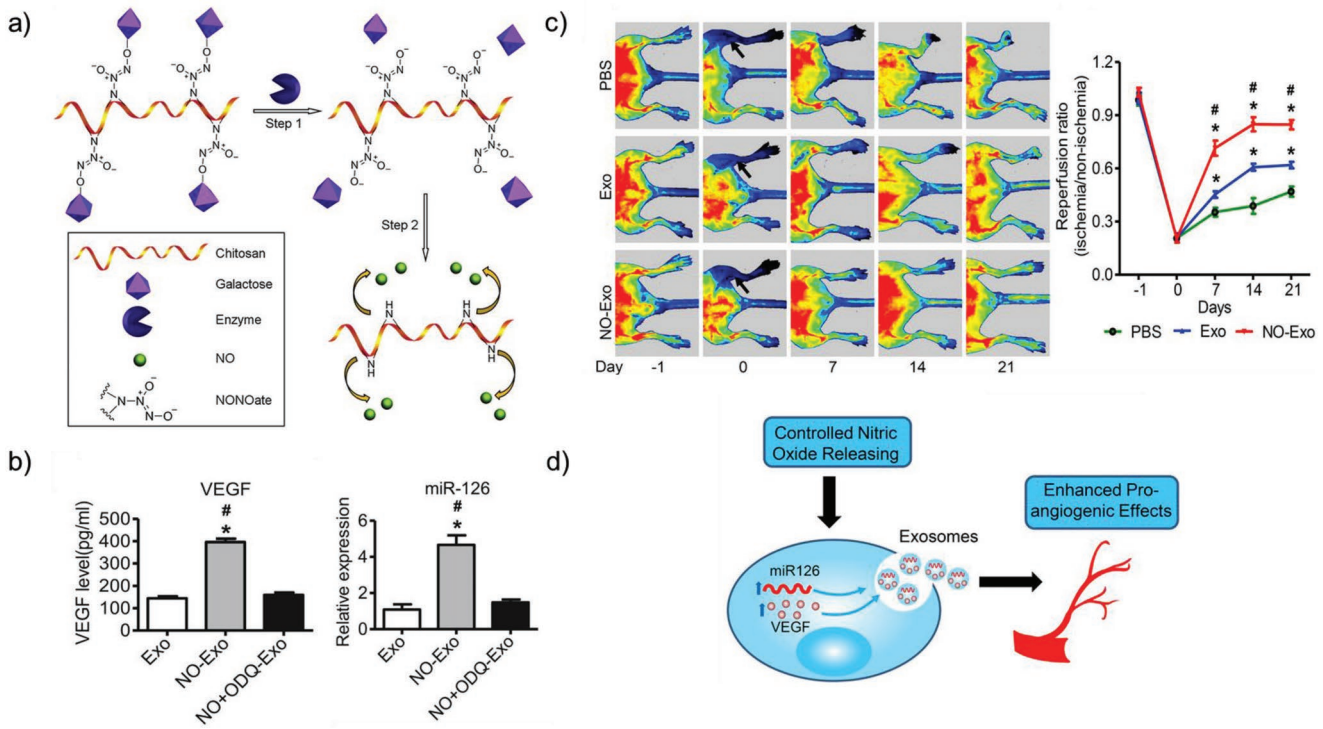

Figure 2. a) Illustration of the decomposition of CS–NONOate caged by β-galactose (CS-NO) under the catalysis of β-galactosidase enzyme and resulting in the controlled release of NO. b) Treatment with NO-induced exosomes resulted in increased synthesis of VEGF and miR-126 by hP-MSCs. c) Blood perfusion in ischemic/healthy limb was detected preoperation and postoperation across 21 days (blood flow increases from dark blue to red). Quantitative analysis is shown alongside, with NO-induced exosomes showing significant improvements from day 7 onward. d) Schematic diagram depicts proangiogenic effects of NO-induced exosome release by hP-MSCs. NO enhanced the levels of VEGF and miR-126 detectable within exosomes. a) Reproduced with permission.[58] Copyright 2013, Elsevier. b–d) Reproduced with permission.[49] Copyright 2017, Elsevier.

DNA encoding eNOS and ADSCs, for combined gene and stem cell therapy treatment of MI. Treatment with these hydrogels resulted in sustained NO delivery to the myocardium and maintained a regenerative microenvironment over 28 days, resulting in reduced infarction size, restricted fibrosis, and significant improvement of heart function. Injectable hydrogels that form gels in situ can be effective means to treat pathological tissues. For example, upon intramyocardial injection and gelation, hydrogels can provide structural support for the weakened myocardial wall and enhance ventricular wall compliancy, while providing a platform for the controlled delivery of drugs or biofactors. However, despite hydrogels having advantageous versatility in the range of treatment options, when used alone they lack cell population-specific delivery and require invasive surgical procedures for implantation.

3.1.2. Microspheres and Nanoparticles

Polymeric microspheres have been widely used in biomedical research due to their controllable size and stability. Microspheres also offer the capacity to package multiple bioactive factors into structures that can mimic 3D spheroid cell microenvironments. A recent example of functionalized NO-releasing microspheres that incorporated 3D stem cell cultures, was shown in an investigation by Regmi et al.[56] Polyethylenimine (PEI)/NONOate was incorporated into poly(lactic-co-glycolic acid) (PLGA) microspheres to deliver NO by diffusion for an extended period of 10 days to in vitro gingiva-derived MSCs (GMSCs) cultures. The researchers then created a hybrid aggregate of GMSCs and NO-releasing microspheres, prepared by the hanging drop technique, which resulted in homogeneous arrangements of GMSCs and microspheres within heterospheroids. NO maintained sustained release over 10 days, effectively improved osteogenic differentiation of GMSCs and accelerated their proliferation. The wider implications of this method are evident when treatments require a tunable and scaffold-free solution, readily adjusted by polymer used, the concentration of NO donor loaded, and the type of stem cells aggregated with microspheres into heterospheroids.

Light-induced NO release from macromolecular assemblies has been achieved by immobilizing photosensitive NO donors on the surfaces or within biomaterials; alternatively, the photosensitive NO donors themselves can be assembled into 3D porous networks. The ability to deliver NO to cells under spatiotemporal control was achieved using an NIR-light-driven NO release nanoplatform, based on upconversion nanoparticles (UCNPs) and light-sensitive NO precursor Roussin's black salt (RBS).[57] The UCNPs were selectively internalized into cancer stem-like cells (CSCs). Excitation by a NIR laser achieved the necessary cell penetration for photolysis of RBS and on-demand release of NO, while avoiding overheating. NO release within CSCs-induced apoptosis by increasing drug sensitivity and declined drug efflux of combined chemotherapy, resulting

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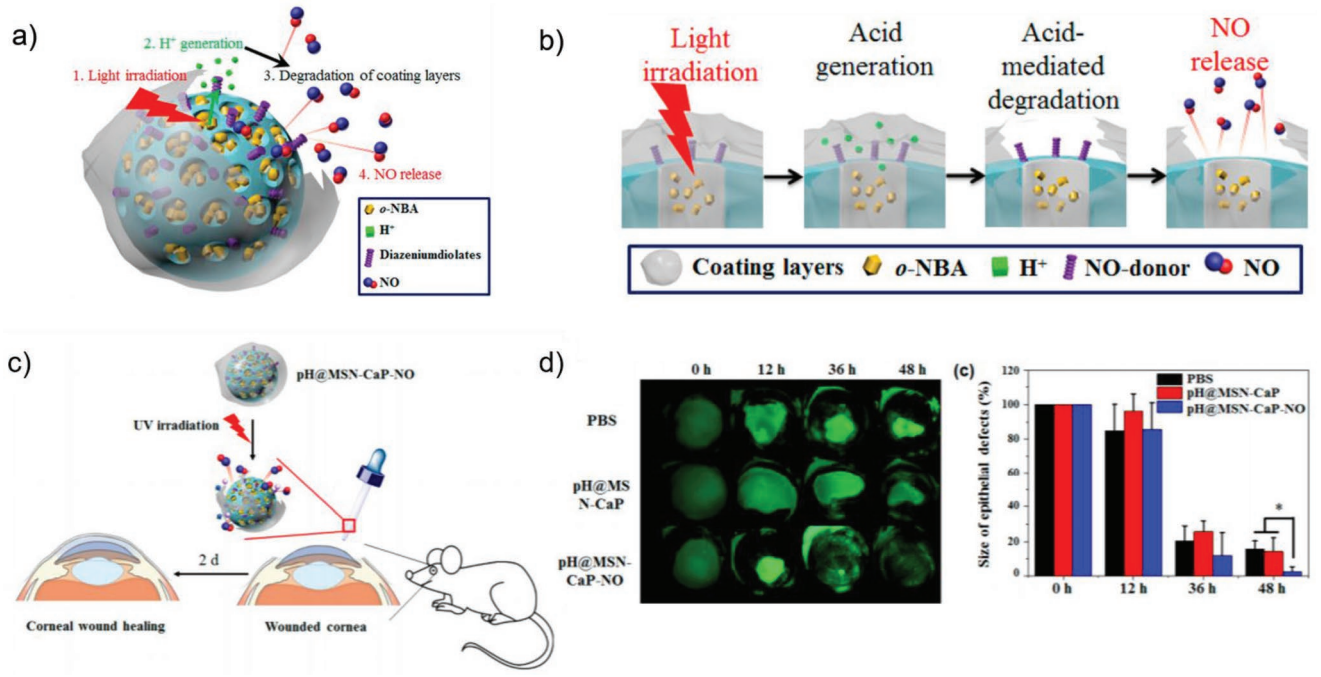

Figure 3. a) Schematic illustration of the designed light-responsive and smart NO-releasing nanoparticles. The gatekeeper is composed of a pH-jump reagent (2-nitrobenzaldehyde, o-NBA) loaded in mesoporous nanoparticles (MSNs) as an intermediary of stimulus, and a calcium phosphate (CaP) coating as a shielding layer for NO release by diazeniumdiolate (pH@MSN-CaP-NO). Light irradiation and subsequent acid generation are the triggers for uncapping the CaP coating gatekeeper. b) Schematic of the light-responsive gatekeeper for smart NO delivery system, and action of release. c) Schematic description of in vivo corneal wound-healing experiments. d) Representative images of corneal wounds treated with light-exposed PBS (control), pH@MSN-CaP, and pH@MSN-CaP-NO; quantification is shown alongside. a–d) Reproduced with permission.[61] Copyright 2016, American Chemical Society.

in tumor elimination in mouse models. Using NIR-induced rapid release of intracellular NO at high concentrations would likely impede stem cell growth and proliferation, however adjustment of donor and NO availability may preserve pluripotency by inhibiting lineage commitment. Choi et al.[61] developed a smart NO-delivery system based on mesoporous nanoparticles that use light-irradiation to induce acidic pH degradation of a coating that surrounds the NO donor. Depending on the rate of acid generated, the release of NO can be tightly controlled (**Figure 3**). The researchers demonstrated the application of this smart delivery system in the controlled release of NO for healing of corneal epithelium wounds in mouse models.

Development of NO-generating microspheres and nanoparticles for stem cell directed therapies is still a relatively early form of nanomedicine. Despite research demonstrating how microspheres can provide a self-contained microenvironment that supports encapsulated cell survival, the stimuli-triggered control of NO flux from microspheres remains an unexplored area. This may be due, in part, to limited NO-related functionality for microspheres, especially when nanoparticles currently represent a more effective means of stimuli-regulated and site-specific NO release. Perhaps unsurprisingly, the majority of NO nanogenerator particles have been employed for use in anticancer settings whereby large NO payload release and cytotoxicity is preferential. For nanoparticle translation to NO therapies for tissue regeneration, factors such as surface charge, systemic administration and circulation, targeted localization, half-life, routes of elimination, and controlled cellular uptake still require optimization.

3.1.3. Surface Coatings

Biomaterial surface coatings offer a biomimetic approach for NO release, which can be applied to a wide range of supporting structures, most commonly dressings, stents, and metallic frameworks. An NO-releasing bioinspired multifunctional nanomatrix coatings consisting of self-assembled peptide amphiphiles were developed to mimic the release of NO by native endothelium.[53] Peptide chains contained endothelial progenitor cell (EPC) recruiting motifs (YIGSR) and matrix metalloproteinase-2 (MMP2) degradable sequences (GTAGLIGQ) linked to a polylysine NO donor sequence (KKKKK). The YIGSR motif and NO release recruited EPCs and modulated their differentiation into ECs, while the MMP2 degradable sequence allowed for migration of EPCs through the nanomatrix. Furthermore, new and facile methods of generating polymer coatings with the capacity to release NO have been developed.[62] Novel implant coatings were formulated by plasma polymerization and not only had bacteriostatic effects, but low cytotoxicity to MSCs and stromal cells.

Our research group coated CS-(β-Gal-NONOate) onto electrospun PCL mats to provide mechanically sound hybrid therapeutic dressings, which maximized the surface area of the wound site exposed to NO release for enhanced therapeutic

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efficacy.[63] While this is a similar strategy to our CS-(β-Gal-NONOate) hydrogel for wound healing, this adaptive methodology demonstrates the versatility of polymer-conjugated donor/surface material combinations, in this case to create a single biomaterial dressing better suited to the intended medical application. Moreover, the NO payload and rate of release in response to enzyme could be readily controlled by altering the ratio of PCL to CS-(β-Gal-NONOate).

Most NO-generating surface coatings have yet to be used for stem cell therapies. Understandably, surface-coated materials are aimed at cardiovascular applications. Yang et al. proposed a strategy that utilized circulating RSNO molecules from blood itself for a local and sustained delivery of NO to vascular stent sites.[64] The team incorporated glutathione peroxidase (GPx) at the solid interface via co-immobilization of selenocystamine (SeCA) in the framework of polydopamine (DA). SeCA/Dopa coatings enabled the development of a catalytic surface for exceptionally long-term local NO-generation over 60 days. A high SeCA/DA molar ratio resulted in an increased NO flux, and range could be controlled to mimic the natural endothelium environment. After continuous exposure to NO donor solution for 60 days, the SeCA/DA coatings still maintained 55% of their NO production rate, indicating long-term efficacy as a coating for blood contacting devices.

Metal–organic frameworks (MOFs) represent a new class of coordination materials created from metal bridged by organic bridging ligands. Yang et al. further improved vascular stent NO production capacity in a recent study that employed Cu^{II}, which has GPx-like NO catalytic activity, to fabricate copper–phenolic–amine MOFs as NO-generating surfaces.[65] Cu^{II} was immobilized onto the stent surface using a one-step metal–catecholamine assembling strategy, forming DA-Cu^{II} MOF complexes on cardiovascular stent surfaces. This facile surface engineering strategy is inspired by the adhesion and protein cross-linking chemistry of $Fe(DA)_3$ complexes found in mussel shell coatings. This was the first report of functional cardiovascular stent coatings, which are combined with in situ NO catalysis by Cu^{II} under physiological pH. NO release rates could be directly modified to natural endothelium rates by adjusting the dose of Cu^{II} immobilized on the surface. After 30 days in solution, NO flux from DA-Cu^{II} coatings remained as high as 72%. Such Cu^{II}-containing coatings on blood-contacting devices exhibited admirable biocatalytic performance and enabled the continuous, controllable, and local conversion of endogenous RSNOs to active NO. Zhao et al.[66] produced surface MOFs from Cu^{II} benzene-1,3,5-tricarboxylate (CuBTC) coatings, which were deposited using layer-by-layer self-assembly methodology on an alkali-activated titanium wire surfaces. Flux of NO release in response to circulating GSNO increased with more deposition cycles of CuBTC coatings. Coatings could induce EC proliferation, whereas 10 cycles of coatings efficiently suppressed smooth muscle cells and macrophages, inhibited platelet adhesion and platelet activation, and attenuated the inflammatory response of titanium after implantation. Cu-based MOFs can serve as effective approaches to generate NO at the surface of stents and could be further utilized for the surface modification of cardiovascular biomaterials to prevent thrombosis and hyperplasia.

Overall, surface coatings for the generation of NO have unrecognized potential for use in stem cell therapies. Functionalization of vascular grafts with NO-generating moieties could assist in the recruitment of stem cells or progenitor cells. Indeed, modified stent surfaces have been generated for the capture and recruitment of circulating stem cells or progenitor cells, such as with the addition of murine antihuman anti-CD34 monoclonal antibodies. Implantation of CD34- stents induced inflammatory responses, and induced migration and proliferation of SMCs as well as the adhesion of blood platelets, leading to restenosis, thrombosis, and delayed reendothelialization.[67] This was proposed to be due to the non-specificity of anti-CD34 to capture endothelial lineage-fated EPCs, as $CD34^+$ cells were also shown to be able to differentiate into SMCs and macrophages.[68] Clinical performance of these vascular stents could be improved with the inclusion of bioactive factors such as NO donors, to modulate the behavior of EPCs toward EC lineages, while inhibiting SMC differentiation.[65] Furthermore, anti-CD133 antibody was identified as a candidate for EPC-specific capture. Immobilization of anti-CD133 antibodies onto the heparin/collagen multilayered ePTFE grafts achieved antithrombotic and rapid endothelialization results.[68] Combination of anti-CD133 and NO can be speculated to a further enhanced effect on graft recruitment of EPCs and vascular regeneration. NO induced S-nitrosylation and subsequent oxidation of thiol groups in the MMP9 prodomain has been suggested to initiate enzyme activation.[69] A crucial event for stem cell and progenitor mobilization is the release of soluble c-Kit ligand by MMP9,[70] a factor abundant within vascular EC populations. Thus, NO generation by EPC-capturing grafts/stents may temper the stem cell/EPC microenvironment response toward elevated vascular regeneration.

3.2. Enzyme–Prodrug Therapy

Enzyme–prodrug therapy (EPT) is a versatile and exploitable technique to release NO (NO-EPT) at the site of action when prodrugs are systemically administrated. Advantages of EPT include targeted and localized delivery, tunability of dosage, duration, and the method of administration.[20] Thus, there is tangible and massive potential for the use of NO-EPT in stem cell co-therapy. We have previously used EPT to fabricate functionalized vascular grafts for assessment as rat abdominal aorta replacements. Galactosidase enzymes were immobilized onto the grafts through avidin–biotin affinity binding.[71] When glycosylated NONOate prodrug was administrated by tail vein injection, it circulated until contact with the graft; the enzyme-immobilized on the vascular grafts catalyzed the decomposition of the NO prodrug, and NO was locally released. This promoted endothelialization and tissue regeneration, owing to the localized and on-demand NO release.[71] Moreover, in vivo stability of the of the functionalized grafts was assessed by subcutaneous implantation over 30 days. The grafts demonstrated only marginally decreased catalytic properties, ascribed to macrophage-mediated degradation and elimination. These results suggest that grafts remained biologically active for up to 1 month, thus it is feasible for NO to be administered within this timeframe, catering to requirement.

Chandrawati et al.[20] published an example of NO-EPT that combined biomaterials to engineer improved release kinetics. In

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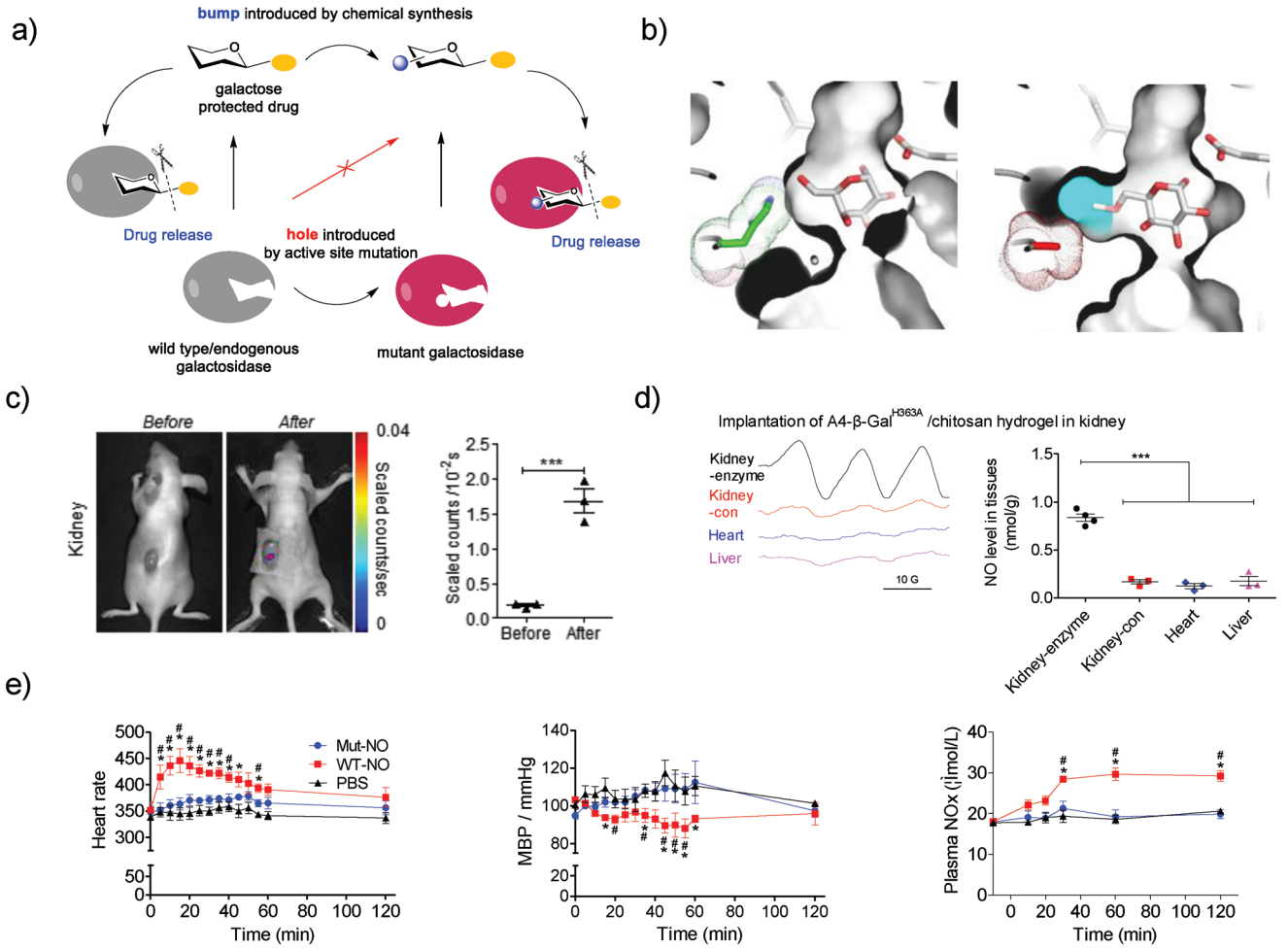

Figure 4. a) Schematic illustration of the "bump-and-hole"-based enzyme (β-galactosidase)–prodrug (galactosyl drug) system. b) Active sites of A4-β-Gal (left) and the engineered A4-β-GalH363A (right) mutant with Sybyl simulation of Gal and MeGal docking with active sites, respectively. c) In vivo bioluminescence imaging (BLI) of the specific and efficient hydrolysis of MeGal-DDAO. d) Targeted NO delivery was evaluated by comparison of NO level in kidney of mice after administration of the A4-β-GalH363A–MeGal-NO pair in kidney model. Shown are EPR spectra of extracts from kidney of mice (left) and NO production in various tissues (right). The amount of NO-Fe(DETC)$_2$ was calibrated using TEMPO as a standard. e) Comparison of the effect of Mut-NO and WT-NO on the heart rate, blood pressure, and plasma NO$_x$ of rats following intraperitoneal injection at a dose of 5 mg kg^{-1}. a–e) Reproduced with permission.[73] Copyright 2019, Springer Nature.

one approach, β-galactosidase enzymes were encapsulated within layer-by-layer assembled poly(methacrylic acid) (PMA) capsules and were subsequently embedded within the trabecular meshwork of the eye for glaucoma therapy. Liposomes containing β-Gal-NONOate were delivered to the aqueous humor outflow pathway. Upon liposome breakdown, slow release of NO donor into the outflow resistance sites underwent enzyme biocatalysis to produce NO. By utilizing a spatial separation and encapsulation of enzyme and prodrug into different microparticles, the half-life of NO release was increased 30-fold, extending release t½ from ≈5 min to ≈2.5 h. The same research team demonstrated an innovative method for incorporating 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes loaded with β-glucuronidase into electrospun poly(vinyl alcohol) (PVA) nonwoven fibers for EPT.[72] Thus, illustrating a strategy that provides a highly sought-after solution for the stabilization of enzymes and protection against proteolytic degradation and loss of activity.

Overcoming non-specific release of NO from prodrugs by biocatalysis from endogenous and circulating enzymes is an important objective in cardiovascular tissue regeneration, as non-specific systemic release of NO may lead to compromising side effects such as heart rate acceleration and blood pressure reduction. In this regard, we developed an alternative strategy in which native enzymes are transformed into mutant homologs, for precise and specific interaction with its corresponding prodrug, eliminating undesired production of NO.[73] Introduction of a methylation "bump" at the O6 position of galactosyl of Gal-NONOate abolished recognition by endogenous β-galactosidase. A mutant β-galactosidase (A4-β-GalH363A) was developed to recognize the 6-methyl-galactose-conjugated NONOate (MeGal-NO). This was achieved by introducing a "hole" within the active site to accommodate the 6-methyl group. We termed this novel approach, the "bump and hole" enzyme–prodrug pair EPT (**Figure 4**). As a result, MeGal-NO improved its own circulation stability and released NO specifically from

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catalysis by A4-β-GalH363A. In situ implantation of CS hydrogels loaded with A4-β-GalH363A at target sites, such as within ischemic hind limbs or kidneys, was followed by intravenous injection of MeGal-NO. Data indicated that NO generated in the targeted locations containing the A4-β-GalH363A/CS hydrogel had highly specific localized release.

Utilization of in situ catalysis of NO release means that NO prodrugs could be administered for specific spatial and temporal release of NO at the site of stem cell implantation, thereby regulating the exposure and effects of NO on stem cell activity, thus maximizing beneficial effects and minimizing side-effects.

4. Conclusions and Outlook

NO-releasing biomaterials have been well represented for anticancer, antimicrobial, and ischemic treatments; the emergence of NO's regulation of stem/progenitor cell function offers exciting implications for the broader applications of NO-based therapies in regenerative medicine. Over recent years, researchers have begun to unveil the ways in which NO stimulation affects stem cell behavior and stem cells adaption of their microenvironment. In turn, these events have significant impact on surrounding resident stromal and immune cells, potentiating the range of NO's therapeutic actions, creating a highly regenerative tissue state, and highlighting the rising roles that NO co-delivery will take in stem cell therapies. The future direction of the broader spectrum of uses for NO-releasing biomaterials is limited by donor parameters for NO release. For stem cell co-therapies, we are currently facing a bottleneck in the development of NO-releasing biomaterials and three factors ultimately determine the material-related strategy for regenerative medicine: 1) biomaterial suitability for the co-delivery of stem cells, maintaining cell survival and prolonging therapeutic effect; 2) extended NO release over the course of the treatment time, with control over on-demand mechanisms of release; and 3) the target tissue site's structure, function, pathology, and accessibility. This has given rise to a new wave of development of "smart donors," to augment payload, stability, and biocompatibility, while improving site-specific release. As novel NO donors are discovered and synthesized, their application in the enhancement of stem-cell-mediated therapies will continue to increase.

Given the clear advantages that hydrogels possess in cell encapsulation and modulation of function and phenotype, they are currently the practical option for combined NO and stem cell therapeutic delivery. Hydrogel-based biomaterials can be tuned to support stem cell encapsulation, adherence, and survival. Choice of hydrogels is situational and based on the regenerative effect required, thus development of intelligently engineered multifaceted hydrogels takes center stage. In the case of replacement of cells lost in pathological and progressive disease development, hydrogels can be engineered to act as biomimetic ECM, providing cues to direct stem cell differentiation into functional lineages and maintaining a level of viscoelasticity to permit freedom of migration out of the gel and into the surrounding host tissue. Should treatments choose to exploit the benefits provided by the stem cell secretome, hydrogels can be engineered to provide stem cell pockets or niches that facilitate exposure to nutrients and NO stimuli while preserving stemness. With the fabrication of more multifunctional and intelligent hydrogels that respond to their contents or external environment, it is likely that hydrogels will shift from the homogeneity to heterogeneity in design, including the incorporation of other biomaterial architecture such as microsphere or nanoparticle generators within their structural bounds. As discussed extensively here, NO can be utilized to potentiate either of these outcomes, based on donor-release mechanisms and NO flux throughout the biomaterial.

In terms of controlled mechanisms of NO release from donors, given the clear advantages of site-specific NO release, freely adjustable payload delivery, tunable rate of release, and long-term stability of enzyme-functionalized materials; NO-EPT using synthetic enzyme–prodrug pairs is a suitable choice for future biomaterial development. There are ways in which NO-EPT could be improved further. In combination with EPT, enzyme-responsive NO prodrug hybrids could overcome clinical problems by enhancing specificity, the potency of low doses of NO, minimizing toxic by-products, and may even provide real-time monitoring of NO-EPT effect over time.

Design and development of new disease-specific NO donors with improved physiochemical properties: higher stability, larger NO donation pools, and improved biocompatibility can provide promising solutions to overcome efficacy concerns of NO-releasing biomaterials. Since the therapeutic efficacy of NO can be precisely controlled by the dosage, high and low doses of NO often lead to utterly opposing effects. This is especially evident in immunoregulation and cancer therapy, and as discussed here, is apparent in stem cells. The desired amount for NO also fluctuates during the various stages of pathophysiological processes. Therefore, the development of microenvironment-responsive NO donors and biomaterials represents a promising direction for future studies. These materials can sense the variations within pathological microenvironments, including glucose, hypoxia, ROS, etc., and decompose to release NO in a controlled and on-demand manner accordingly. Furthermore, as alternatives to monitoring NO release, multifunctional biomaterial delivery systems with both diagnostic and therapeutic functions to visualize the evolution of diseases as well as the fate of stem cells have started to take advantage of advanced imaging technology currently available to researchers.

In summary, a full understanding of NO influence on stem cell behavior, function, and release profiles affecting the tissue microenvironment will lead to the development of functional biomaterials with precise NO release that exploit these effects, emanating minimal side effects and cohesive, accelerated tissue regeneration. As fabrication of smart delivery systems advance, novel NO-releasing biomaterial systems will drive progress in stem cell therapy, providing a new library of clinical solutions for regenerative medicine.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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[1] W. L. Murphy, T. C. McDevitt, A. J. Engler, *Nat. Mater.* **2014**, *13*, 547.
[2] T. Yang, A. N. Zelikin, R. Chandrawati, *Adv. Sci.* **2018**, *5*, 1701043.
[3] F. Bonafe, C. Guarnieri, C. Muscari, *J. Physiol. Biochem.* **2015**, *71*, 141.
[4] R. SoRelle, *Circulation* **1998**, *98*, 2365.
[5] a) J. Lei, Y. Vodovotz, E. Tzeng, T. R. Billiar, *Nitric Oxide* **2013**, *35*, 175; b) M. Tirone, V. Conti, F. Manenti, P. A. Nicolosi, C. D'Orlando, E. Azzoni, S. Brunelli, *PLoS One* **2016**, *11*, e0164893; c) Y. Sagi, M. Heiman, J. D. Peterson, S. Musatov, M. Scarduzio, S. M. Logan, M. G. Kaplitt, D. J. Surmeier, N. Heintz, P. Greengard, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 17636; d) C. Bogdan, *Trends Immunol.* **2015**, *36*, 161; e) S. Kashiwagi, Y. Izumi, T. Gohongi, Z. N. Demou, L. Xu, P. L. Huang, D. G. Buerk, L. L. Munn, R. K. Jain, D. Fukumura, *J. Clin. Invest.* **2005**, *115*, 1816.
[6] a) J. Kim, B. C. Yung, W. J. Kim, X. Chen, *J. Controlled Release* **2017**, *263*, 223; b) Z. Huang, J. Fu, Y. Zhang, *J. Med. Chem.* **2017**, *60*, 7617.
[7] a) A. Khan, H. Dawoud, T. Malinski, *Int. J. Nanomed.* **2018**, *13*, 455; b) B. P. Carreira, D. F. Santos, A. I. Santos, C. M. Carvalho, I. M. Araujo, *Oxid. Med. Cell. Longevity* **2015**, *2015*, 451512.
[8] L. Cai, J. B. Klein, Y. J. Kang, *J. Biol. Chem.* **2000**, *275*, 38957.
[9] a) B. M. Curtis, K. A. Leix, Y. Ji, R. S. Glaves, D. E. Ash, D. K. Mohanty, *Biochem. Biophys. Res. Commun.* **2014**, *450*, 208; b) S. Brunelli, C. Sciorati, G. D'Antona, A. Innocenzi, D. Covarello, B. G. Galvez, C. Perrotta, A. Monopoli, F. Sanvito, R. Bottinelli, E. Ongini, G. Cossu, E. Clementi, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 264; c) Y. Kang, J. Kim, Y. M. Lee, S. Im, H. Park, W. J. Kim, *J. Controlled Release* **2015**, *220*, 624; d) S. P. Hussain, G. E. Trivers, L. J. Hofseth, P. He, I. Shaikh, L. E. Mechanic, S. Doja, W. Jiang, J. Subleski, L. Shorts, D. Haines, V. E. Laubach, R. H. Wiltrout, D. Djurickovic, C. C. Harris, *Cancer Res.* **2004**, *64*, 6849.
[10] S. P. Nichols, W. L. Storm, A. Koh, M. H. Schoenfisch, *Adv. Drug Delivery Rev.* **2012**, *64*, 1177.
[11] C. Oliveira, S. Benfeito, C. Fernandes, F. Cagide, T. Silva, F. Borges, *Med. Res. Rev.* **2018**, *38*, 1159.
[12] A. L. Fitzhugh, L. K. Keefer, *Free Radical Biol. Med.* **2000**, *28*, 1463.
[13] J. S. Stamler, O. Jaraki, J. Osborne, D. I. Simon, J. Keaney, J. Vita, D. Singeli, C. R. Valeri, J. Loscalzo, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7674.
[14] a) S. Hwang, M. E. Meyerhoff, *Biomaterials* **2008**, *29*, 2443; b) P. C. Ford, *Nitric Oxide* **2013**, *34*, 56; c) T. Suzuki, O. Nagae, Y. Kato, H. Nakagawa, K. Fukuhara, N. Miyata, *J. Am. Chem. Soc.* **2005**, *127*, 11720.
[15] a) J. S. Huang, L. Y. Chuang, J. Y. Guh, Y. J. Huang, *Toxicol. Sci.* **2009**, *111*, 109; b) Y. Liu, C. Xia, R. Wang, J. Zhang, T. Yin, Y. Ma, L. Tao, *Clin. Exp. Pharmacol. Physiol.* **2017**, *44*, 854.
[16] a) N. Bao, J. Ou, N. Li, P. Zou, J. Sun, L. Chen, *Eur. J. Med. Chem.* **2018**, *154*, 1; b) K. Tian, F. Xu, X. Gao, T. Han, J. Li, H. Pan, L. Zang, D. Li, Z. Li, T. Uchita, M. Gao, H. Hua, *Eur. J. Med. Chem.* **2017**, *136*, 131.
[17] a) L. V. Tatyanenko, N. Y. Shmatko, N. A. Sanina, O. V. Dobrokhotova, I. Y. Pikhteleva, A. I. Kotel'nikov, S. M. Aldoshin, *Bull. Exp. Biol. Med.* **2017**, *163*, 54; b) D. Maksimovic-Ivanic, P. Fagone, J. McCubrey, K. Bendtzen, S. Mijatovic, F. Nicoletti, *Int. J. Cancer* **2017**, *140*, 1713; c) L. Yang, X. Wang, D. J. Suchyta, M. H. Schoenfisch, *Bioconjugate Chem.* **2018**, *29*, 35.
[18] X. J. Wu, X. P. Tang, M. Xian, P. G. Wang, *Tetrahedron Lett.* **2001**, *42*, 3779.
[19] X. Yao, Y. Liu, J. Gao, L. Yang, D. Mao, C. Stefanitsch, Y. Li, J. Zhang, L. Ou, D. Kong, Q. Zhao, Z. Li, *Biomaterials* **2015**, *60*, 130.
[20] R. Chandrawati, J. Y. H. Chang, E. Reina-Torres, C. Jumeaux, J. M. Sherwood, W. D. Stamer, A. N. Zelikin, D. R. Overby, M. M. Stevens, *Adv. Mater.* **2017**, *29*, 1604932.
[21] X. Jia, Y. Zhang, Y. Zou, Y. Wang, D. Niu, Q. He, Z. Huang, W. Zhu, H. Tian, J. Shi, Y. Li, *Adv. Mater.* **2018**, *30*, 1704490.
[22] J. Hou, H. He, S. Huang, M. Qian, J. Wang, X. Tan, G. Han, Y. Song, Z. Xu, Y. Liu, *Chem. Commun.* **2019**, *55*, 1205.
[23] R. Guo, Y. Tian, Y. Wang, W. Yang, *Adv. Funct. Mater.* **2017**, *27*, 1606398.
[24] M. Blangetti, A. Fraix, L. Lazzarato, E. Marini, B. Rolando, F. Sodano, R. Fruttero, A. Gasco, S. Sortino, *Chem. - Eur. J.* **2017**, *23*, 9026.
[25] N. Sachewsky, C. M. Morshead, *Stem Cells Dev.* **2014**, *23*, 2469.
[26] H. Chen, X. H. Min, Q. Y. Wang, F. W. Leung, L. Shi, Y. Zhou, T. Yu, C. M. Wang, G. An, W. H. Sha, Q. K. Chen, *Sci. Rep.* **2015**, *5*, 8718.
[27] a) K. Mujoo, J. S. Krumenacker, F. Murad, *Free Radical Biol. Med.* **2011**, *51*, 2150; b) Y. Nie, K. Zhang, S. Zhang, D. Wang, Z. Han, Y. Che, D. Kong, Q. Zhao, Z. Han, Z. X. He, N. Liu, F. Ma, Z. Li, *Acta Biomater.* **2017**, *63*, 190.
[28] B. P. Carreira, M. I. Morte, A. Inacio, G. Costa, J. Rosmaninho-Salgado, F. Agasse, A. Carmo, P. Couceiro, P. Brundin, A. F. Ambrosio, C. M. Carvalho, I. M. Araujo, *Stem Cells* **2010**, *28*, 1219.
[29] M. Bergsland, R. Covacu, C. Perez Estrada, M. Svensson, L. Brundin, *Stem Cells* **2014**, *32*, 2539.
[30] S. Yang, L. Guo, Y. Su, J. Wen, J. Du, X. Li, Y. Liu, J. Feng, Y. Xie, Y. Bai, H. Wang, Y. Liu, *Stem Cell Res. Ther.* **2018**, *9*, 118.
[31] A. Nogueira-Pedro, C. C. Dias, H. Regina, C. Segreto, P. C. Addios, L. Lungato, V. D'Almeida, C. C. Barros, E. M. Higa, M. V. Buri, A. T. Ferreira, E. J. Paredes-Gamero, *Stem Cells* **2014**, *32*, 2949.
[32] G. Pan, J. A. Thomson, *Cell Res.* **2007**, *17*, 42.
[33] R. Tapia-Limonchi, G. M. Cahuana, E. Caballano-Infantes, C. Salguero-Aranda, A. Beltran-Povea, A. B. Hitos, A. Hmadcha, F. Martin, B. Soria, F. J. Bedoya, J. R. Tejedo, *J. Cell. Biochem.* **2016**, *117*, 2078.
[34] a) F. Spallotta, J. Rosati, S. Straino, S. Nanni, A. Grasselli, V. Ambrosino, D. Rotili, S. Valente, A. Farsetti, A. Mai, M. C. Capogrossi, C. Gaetano, B. Illi, *Stem Cells* **2010**, *28*, 431; b) J. R. Tejedo, R. Tapia-Limonchi, S. Mora-Castilla, G. M. Cahuana, A. Hmadcha, F. Martin, F. J. Bedoya, B. Soria, *Cell Death Dis.* **2010**, *1*, e80.
[35] Y. Wang, Z. C. Li, P. Zhang, E. Poon, C. W. Kong, K. R. Boheler, Y. Huang, R. A. Li, X. Yao, *Stem Cells* **2015**, *33*, 2973.
[36] P. B. Massion, O. Feron, C. Dessy, J. L. Balligand, *Circ. Res.* **2003**, *93*, 388.
[37] S. Ghannam, C. Bouffi, F. Djouad, C. Jorgensen, D. Noel, *Stem Cell Res. Ther.* **2010**, *1*, 2.
[38] G. Ren, L. Zhang, X. Zhao, G. Xu, Y. Zhang, A. I. Roberts, R. C. Zhao, Y. Shi, *Cell Stem Cell* **2008**, *2*, 141.
[39] Y. Shi, J. Su, A. I. Roberts, P. Shou, A. B. Rabson, G. Ren, *Trends Immunol.* **2012**, *33*, 136.
[40] M. M. Duffy, T. Ritter, R. Ceredig, M. D. Griffin, *Stem Cell Res. Ther.* **2011**, *2*, 34.

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[41] S. Kanno, P. K. Kim, K. Sallam, J. Lei, T. R. Billiar, L. L. Shears 2nd, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12277.
[42] K. Mujoo, V. G. Sharin, N. S. Bryan, J. S. Krumenacker, C. Sloan, S. Parveen, L. E. Nikonoff, A. Y. Kots, F. Murad, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18924.
[43] H. Ding, K. C. Keller, I. K. Martinez, R. M. Geransar, K. O. zur Nieden, S. G. Nishikawa, D. E. Rancourt, N. I. zur Nieden, *J. Cell Sci.* **2012**, *125*, 5564.
[44] S. Sonoda, Y. F. Mei, I. Atsuta, A. Danjo, H. Yamaza, S. Hama, K. Nishida, R. Tang, Y. Kyumoto-Nakamura, N. Uehara, T. Kukita, F. Nishimura, T. Yamaza, *Sci. Rep.* **2018**, *8*, 3419.
[45] N. Cordani, V. Pisa, L. Pozzi, C. Sciorati, E. Clementi, *Stem Cells* **2014**, *32*, 874.
[46] H. He, Y. Liu, Z. Zhou, C. Guo, H. Y. Wang, Z. Wang, X. Wang, Z. Zhang, F. G. Wu, H. Wang, D. Chen, D. Yang, X. Liang, J. Chen, S. Zhou, X. Liang, X. Qian, Y. Yang, *Free Radical Biol. Med.* **2018**, *123*, 1.
[47] S. A. Gomes, E. B. Rangel, C. Premer, R. A. Dulce, Y. Cao, V. Florea, W. Balkan, C. O. Rodrigues, A. V. Schally, J. M. Hare, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 2834.
[48] N. F. Huang, F. Fleissner, J. Sun, J. P. Cooke, *Stem Cells Dev.* **2010**, *19*, 1617.
[49] W. Du, K. Zhang, S. Zhang, R. Wang, Y. Nie, H. Tao, Z. Han, L. Liang, D. Wang, J. Liu, N. Liu, Z. Han, D. Kong, Q. Zhao, Z. Li, *Biomaterials* **2017**, *133*, 70.
[50] J. Zhang, H. Qi, H. Wang, P. Hu, L. Ou, S. Guo, J. Li, Y. Che, Y. Yu, D. Kong, *Artif. Organs* **2006**, *30*, 898.
[51] Q. Xing, K. Yates, A. Bailey, C. Vogt, W. He, M. C. Frost, F. Zhao, *Surf. Innovations* **2013**, *1*, 224.
[52] W. Wang, B. Tan, J. Chen, R. Bao, X. Zhang, S. Liang, Y. Shang, W. Liang, Y. Cui, G. Fan, H. Jia, W. Liu, *Biomaterials* **2018**, *160*, 69.
[53] A. Andukuri, Y. D. Sohn, C. P. Anakwenze, D. J. Lim, B. C. Brott, Y. S. Yoon, H. W. Jun, *Tissue Eng., Part C* **2013**, *19*, 375.
[54] Q. Xing, L. Zhang, T. Redman, S. Qi, F. Zhao, *J. Biomed. Mater. Res., Part A* **2015**, *103*, 3807.
[55] S. McIlhenny, P. Zhang, T. Tulenko, J. Comeau, S. Fernandez, A. Policha, M. Ferroni, E. Faul, G. Bagameri, I. Shapiro, P. DiMuzio, *J. Tissue Eng. Regener. Med.* **2015**, *9*, 1277.
[56] S. Regmi, J. Cao, S. Pathak, B. Gupta, B. Kumar Poudel, P. T. Tung, S. Yook, J. B. Park, C. S. Yong, J. O. Kim, J. W. Yoo, J. H. Jeong, *Int. J. Pharm.* **2017**, *520*, 163.
[57] X. Zhang, Z. Guo, J. Liu, G. Tian, K. Chen, S. Yu, Z. Gu, *Sci. Bull.* **2017**, *62*, 985.
[58] Q. Zhao, J. Zhang, L. Song, Q. Ji, Y. Yao, Y. Cui, J. Shen, P. G. Wang, D. Kong, *Biomaterials* **2013**, *34*, 8450.
[59] a) Y. Deng, G. Chen, M. Ye, Y. He, Z. Li, X. Wang, C. Ou, Z. Yang, M. Chen, *J. Biomed. Nanotechnol.* **2018**, *14*, 1458; b) G. Chen, J. Li, M. Song, Z. Wu, W. Zhang, Z. Wang, J. Gao, Z. Yang, C. Ou, *Adv. Funct. Mater.* **2017**, *27*, 1701798.
[60] J. Gao, W. Zheng, J. Zhang, D. Guan, Z. Yang, D. Kong, Q. Zhao, *Chem. Commun.* **2013**, *49*, 9173.
[61] H. W. Choi, J. Kim, J. Kim, Y. Kim, H. B. Song, J. H. Kim, K. Kim, W. J. Kim, *ACS Nano* **2016**, *10*, 4199.
[62] T. D. Michl, B. R. Coad, M. Doran, M. Osiecki, M. H. Kafshgari, N. H. Voelcker, A. Husler, K. Vasilev, H. J. Griesser, *Chem. Commun.* **2015**, *51*, 7058.
[63] X. Zhou, H. Wang, J. Zhang, X. Li, Y. Wu, Y. Wei, S. Ji, D. Kong, Q. Zhao, *Acta Biomater.* **2017**, *54*, 128.
[64] Z. Yang, Y. Yang, L. Zhang, K. Xiong, X. Li, F. Zhang, J. Wang, X. Zhao, N. Huang, *Biomaterials* **2018**, *178*, 1.
[65] F. Zhang, Q. Zhang, X. Li, N. Huang, X. Zhao, Z. Yang, *Biomaterials* **2019**, *194*, 117.
[66] Q. Zhao, Y. Fan, Y. Zhang, J. Liu, W. Li, Y. Weng, *ACS Appl. Mater. Interfaces* **2019**, *11*, 7872.
[67] B. Cui, L. Huang, Y. Fang, R. Guo, Y. Yin, X. Zhao, *Microvasc. Res.* **2011**, *81*, 143.
[68] S. Lu, P. Zhang, X. Sun, F. Gong, S. Yang, L. Shen, Z. Huang, C. Wang, *ACS Appl. Mater. Interfaces* **2013**, *5*, 7360.
[69] Z. Gu, M. Kaul, B. Yan, S. J. Kridel, J. Cui, A. Strongin, J. W. Smith, R. C. Liddington, S. A. Lipton, *Science* **2002**, *297*, 1186.
[70] B. Heissig, K. Hattori, S. Dias, M. Friedrich, B. Ferris, N. R. Hackett, R. G. Crystal, P. Besmer, D. Lyden, M. A. Moore, Z. Werb, S. Rafii, *Cell* **2002**, *109*, 625.
[71] Z. Wang, Y. Lu, K. Qin, Y. Wu, Y. Tian, J. Wang, J. Zhang, J. Hou, Y. Cui, K. Wang, J. Shen, Q. Xu, D. Kong, Q. Zhao, *J. Controlled Release* **2015**, *210*, 179.
[72] R. Chandrawati, M. T. J. Olesen, T. C. C. Marini, G. Bisra, A. G. Guex, M. G. de Oliveira, A. N. Zelikin, M. M. Stevens, *Adv. Healthcare Mater.* **2017**, *63*, 80.
[73] J. Hou, Y. Pan, D. Zhu, Y. Fan, G. Feng, Y. Wei, H. Wang, K. Qin, T. Zhao, Q. Yang, Y. Zhu, Y. Che, Y. Liu, J. Cheng, D. Kong, P. G. Wang, J. Shen, Q. Zhao, *Nat. Chem. Biol.* **2019**, *15*, 151.

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