MINI REVIEW

Nanotechnology and stem cells in vascular biology

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Abstract

Nanotechnology and stem cells are one of the most promising strategies for clinical medicine applications. The article provides an up-to-date view on advances in the field of regenerative and targeted vascular therapies describing a molecular design (propulsion mechanism, composition, target identification) and applications of nanorobots. Stem cell paragraph presents current clinical application of various cell types involved in vascular biology including mesenchymal stem cells, very small embryonic-like stem cells, induced pluripotent stem cells, mononuclear stem cells, amniotic fluid-derived stem cells and endothelial progenitor cells. A possible bridging between the two fields is also envisioned, where bio-inspired, safe, long-lasting nanorobots can fully target the cellular specific cues and even drive vascular process in a timely manner.

Key Words
▶ stem cells
▶ nanotechnology
▶ nanorobots

Nanotechnology and stem cells hold a great clinical promise in the field of vascular therapies. Presented review provides an up-to-date summary about advances and challenges associated with these novel treatment strategies.

Nanotechnology systems

The application of nanotechnology in medicine (nanomedicine) has spawned a galaxy of tools with diagnostic, therapeutic, or ideally theragnostic abilities (1). Nanorobotics, in particular, holds a future yet promising potential to change the panorama of fields like cardiovascular intervention, neurological and cancer treatment (2). The devised technologies, relatively to their aim and complexity, face several translational challenges

and attrition (3). Nanoparticles, such as liposomal, polymeric, metallic or their specific combinations are the most investigated and advanced nanotool (in terms of clinical translation). The topic merits an extensive separate description, and the reader is referred to detailed work on the topic (4). We will refer to nanoparticles in the specific contest of load/cargos for nanorobots or specific vascular applications.

Appropriate design of nanorobots design can help diagnosis or accurately deliver payloads, which can be towed or embedded in the structure. Differently from nanoparticles, which are mostly produced and modified in solution-based systems, nanorobotic fabrication also requires the use of technique proper of micro/nano electromechanical systems. Several definitions are possible for a nanorobotics system, both at the scientific and legislative level (e.g. the classification into drug

agent or medical device by the principle of action). A safe definition is an artificial device composed of nanoscale components and up to 10 µm in size (5). This description is not standard and that might explain the various and sometimes misused denomination of nanorobots to bionanotechnological devices.

The presently proposed systems can be classified in terms of (i) propulsion, mechanism, (ii) composition, (iii) target identification, (iv) specific application. Propulsion can be autonomous (chemotaxis, bioseparation) or external (magnetic, acoustic, laser) (6). Although intriguing, autonomous propulsion systems (assimilable to molecular motors) are mostly based on the conversion of glucose into hydrogen peroxide (7), raising a concern about the reactive-oxygen species toxicity of such nanotools. The design, safety and motion freedom are greatly facilitated in externally driven nanorobots, usually referred as nanoswimmers (8). These systems can present intrinsic magnetism or embedded magnetic nanoparticles; specific geometry/material-driven torque formation when exposed to stimuli; and material-mediated hydrolyzing properties under UV light (for which the in vivo application is debatable) (6). Mixed propulsion systems have also been investigated (9). Nanorobots can be constituted of purely synthetic materials (10), biomolecules (DNA in the form of origami, aptamers) (11), biological entities (e.g. magnetotactic bacteria) (1), or hybrid cellular (e.g. loaded red blood cells, neutrophils) flagellated nanocomposites (12, 13). The target identification employed by these systems may rely on precise localization through focused external driving forces (and this requires precise modeling of the hydrodynamic conditions for different vessel) (14) or on sensed environmental properties and moieties (changes of temperature, pH, osmosis or specific biomarker) (11).

As aforementioned, applications of nanodevices in biological systems cover several and very diversified environments: we will for the scope of this review focus on the application for advancement of vascular intervention. In coronary artery disease, nanotechnological systems have been investigated for its low invasiveness during interventional procedures and drug targeted delivery (15). One instance is use of nano-coated stents for endothelial healing, reendothelialization and anti-restenosis (e.g. with nano-embedded sirolimus, rapamycin, NO-loaded polycaprolactone) (16, 17, 18) or nanofibrous scaffolds for coronary bypass. The nanofabrication involves mimicking moieties (e.g. dimyristoyl phosphatidylcholine for cholesterol removal (15)) specific targeting (e.g. with local high levels of pravastatins (19) or toward inflammatory

monocytes (20)) or clearance prolongation of nanosized drugs (21). The general aim is to provide a long-lasting or permanent implant for resolution of the structural damage in combination with reparative and protective strategies. So far, nanomedicine for vascular therapies is focused mostly on nanoparticles. More specifically to nanorobotic entities, DNA origami and aptamers are extensively studied as antithrombotics and anticoagulants (anti-vWF, factor IX and thrombin) (22).

Another application of nanorobots is the individualization and targeting of microvasculature not accessible with conventional techniques (5). Notable examples include the cerebral vasculature and the network of small tumoral masses that escape resection or conventional therapies. Nanorobots have been indeed proposed to spot brain aneurysm detection (23) and to repair small vessel via Von Willebrand factor (vWF) sensing (24). These systems would greatly aid the interventions on non-navigable or poorly visualizable vasculature. Bacterial systems like non-pathogenic *Escherichia coli* have been proven to respond by chemotaxis to concentration of VEGF, a well-known angiogenic cytokine (25), pointing at the potential applications of natural molecular machinery and chemosensors. On the theragnostic side, DNA nanorobots targeting nucleolin-expression tumoral endothelium has been tested *in vivo*, aiming at the starvation of solid tumors by clotting of feeding microvasculature (26). Appropriately designed robots in terms of low toxicity (multiple doses) and retainment can in principle target or drive small tumors at precise checkpoints, making them more sensitive to drugs or conventional therapies.

Stem cells

Vascular homeostasis depends on endothelium integrity and complex interaction between endothelial cells (ECs), vascular smooth muscle cells, and extracellular matrix (27). Additionally, vascular stem and progenitor cells have been identified in all three layers of the vessel wall (most abundantly in the adventitia) (28).

Recent studies described the effects of heterogeneous cell populations in vascular systems through several mechanisms (paracrine modulation, proliferation, transdifferentiation). This paragraph describes various types of stem cells focusing on current clinical application.

Currently, mesenchymal stem cells (MSCs) are considered as the most promising cell type with a wide range of potential therapeutic applications. MCSs are

present in multiple organs (29) throughout human body including adipose tissue (adipose-derived stromal/stem cells, ASCs) and umbilical cord, which provides an easily accessible and expandable source of cells for clinical use (30, 31). Furthermore, MSCs possess particular characteristics: (i) strong angiogenic and paracrine potential, (ii) ability to differentiate to vascular cells contributing to angio- and arteriogenesis (32), (iii) favorable immunogenic profile opening potential for allogenic source (33). Laboratory findings and animal models (34) were confirmed in early phase clinical trials, where autologous ASCs have been showed to provide beneficial clinical effects in patients with critical limb ischemia (35). Moreover, recent myocardial infarction (MI) studies confirmed a safety profile of transendocardial (36) and intracoronary (37) application of allogenic MSCs, which resulted in increased left ventricle ejection fraction (LVEF) and stroke volume. Similarly, meta-analysis including 12 studies showed MSCs to be safe in patients with ischemic heart failure (38).

Other supportive cell candidates can be found in selected but more restricted pools. Amniotic fluid stem cells (AFCS) showed in preclinical studies the possibility of differentiating into vascular cell lineages (39). Moreover, an ongoing clinical trial (NCT03899298, https://clinicaltrials.gov) evaluates safety and efficacy of AFSCs and umbilical cord stem cells for treatment of a broad spectrum of diseases including neurologic, cardiac and pulmonary conditions. Mononuclear cells (MNCs) represent heterogenic population of stem/progenitor cells including hematopoietic stem cells, endothelial progenitor cells (EPCs) and MSCs. Despite the wide application of MNCs in clinical trials for nonhematopoietic tissue regeneration, the recent meta-analyses showed that MNCs do not improve outcomes in patients with peripheral artery disease (40, 41, 42) and acute MI (43). Finally, very small embryonic-like stem cells (VSELs) are small-sized cells (≈5–7 µm in humans) present in adult tissues with an ability to differentiate into three germ lineages *in vitro* (including endothelial progenitor) without manipulation with DNA vectors (44, 45). Moreover, mobilization of VSELs was reported in humans after MI. Nevertheless, it must be noted that VSELs are a rare cell type; thus, sufficient expansion must be obtained for clinical application (44).

A separate outlook is due when considering differentiation-mediated cell therapy, by using human-induced pluripotent stem cells (hiPSCs). These hold a great promise for regenerative medicine (ability to differentiate into ≈200 cell types) (45), but present many challenges

to overcome prior to clinical step. hiPSCs can be derived from patient-specific somatic cells (i.e. fibroblast), and they can be differentiated into ECs (46), which express angiogenic and reendothelialization potential upon exposure to shear stress (46, 47).

Despite promising results in preclinical studies (48), the main limitations for human application include hiPSCs tumorigenicity (49), further transdifferentiation into mature cell type, genomic instability, and risk of mitochondrial mutations (45). To circumvent safety challenges, the novel approaches are incorporated to eliminate the risk of genomic modifications via miRNAs, recombinant proteins and small molecules (50, 51). Presented strategies have a chance to unlock tremendous clinical potential of iPSCs.

A second cell-based approach to vascular therapy can be directly related to resident and circulating EPCs, which do not require passing through differentiation processes. In the light of ongoing scientific debate (52), EPCs of hematopoietic origin are most commonly defined by CD133+/CD34+/vascular endothelial growth factor receptor 2 (VEGFR2+) surface markers (53), while EPCs from non-hematopoietic tissues (adipose tissue, placenta) express various types of antigens (54). Interestingly, endothelial colony-forming cells (ECFCs), a subtype of peripheral blood (PB) EPCs, originate from vessel wall (55, 56). Supporting endogenous resident ECs in replacing damaged endothelium, EPCs might be involved in endothelial repair process by paracrine effect (57) or contribute to new vessel formation (58) with the presence of late-stage endothelial markers (59). Furthermore, crosstalk between ECs and EPCs is orchestrated by chemoattractant gradient of growth factors, chemokines, and cytokines (60). Despite encouraging experimental and preclinical therapeutic evidence of EPCs role (61), only modest benefits were found at the clinical level for treatment of MI (+3.15% LVEF) (62), critical limb ischemia, refractory angina, and chronic myocardial ischemia (63). Moreover, EPCs expressing bone antigens are found to be involved in arterial wall stiffening and calcification (64, 65).

Conclusions

Drawing a final conclusion about the application of nanotechnology and heterologous stem cell pools in human vascular diseases (summarized on Fig. 1) is currently still challenging due to (i) the large heterogeneity of device designs and broad application scopes;
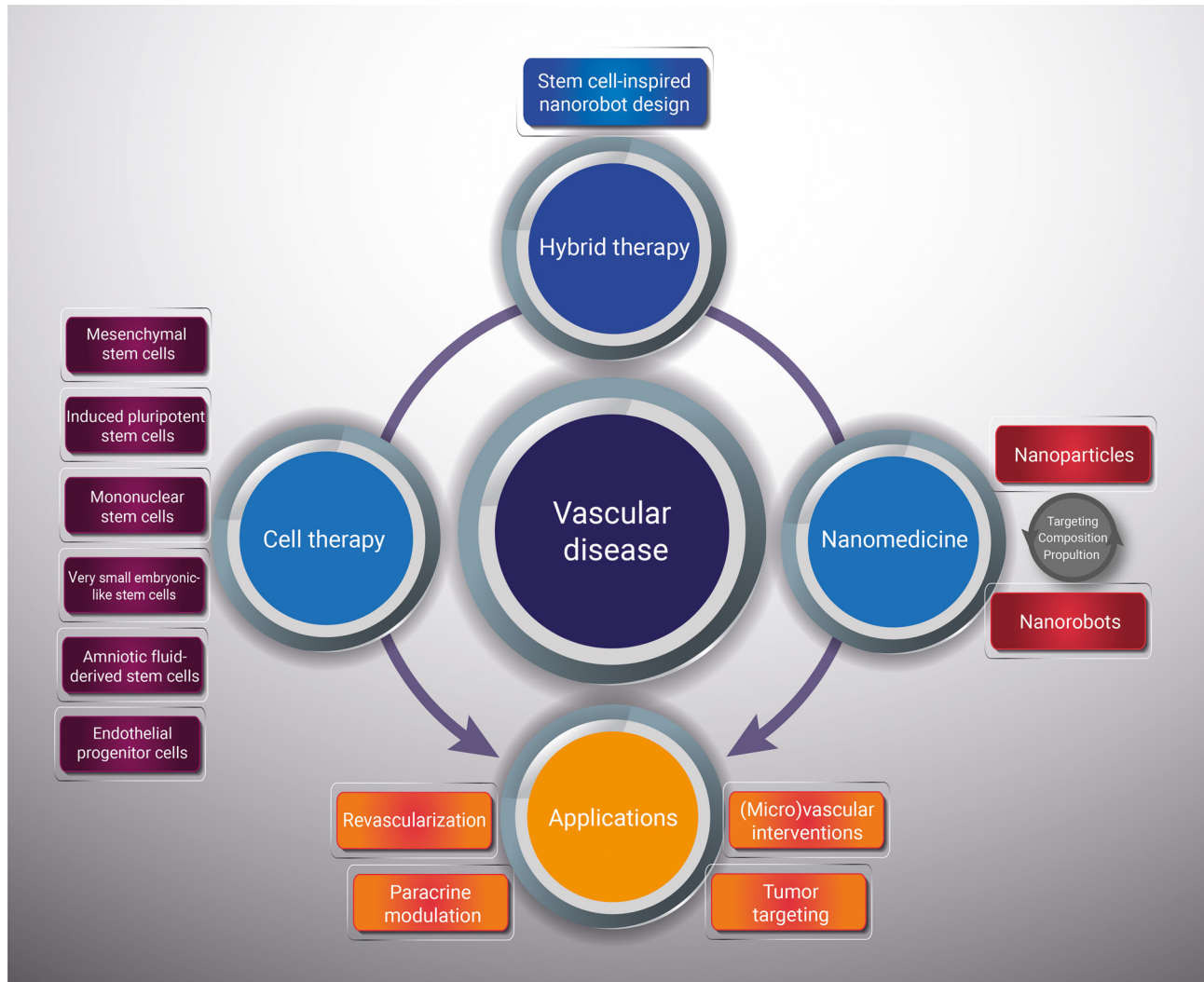
Figure 1
The scope of nanotechnology and stem cell applications in human vascular diseases.

(ii) incomplete cell characterization and administration protocols. The limited follow-up in preclinical and clinical trials leaves open questions common to the two fields, most importantly the grade of systemic biological effect (i.e. oxidative effects of autonomous nanorobots, uncontrolled differentiation cues for stem cells, and organ-specific retention in presence of comorbidities) coming from the exogenous material. Non-invasive assisted imaging modalities of nanorobots are fundamental for the long-term monitoring of the therapy, on the one hand. A failsafe quenching mode (e.g. transient supraphysiological temperature degradation) would be also a useful design feature to avoid overtreatment, on the other. Nevertheless, the promised benefits for the two approaches justify further investigation, with a possible envisioned bridging between them. It is not far-fetched, looking at the state of the art, to imagine highly selective piloted DNA-synthetic nanorobots specifically engineered to recognize paracrine stem/progenitor cells exosome. This could allow precise imaging of the interested area and topical deliver therapeutic cargo (e.g. drugs or differentiative cytokines), possibly in a way that will disable the nanorobot and allow its wasting. A tandem work between nanorobotics design and fate/role of vascular stem cells could boost the efficacy and translation of regenerative and targeted vascular therapies.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Author contribution statement

T J and G C were responsible for article design, literature collection, and drafting of the manuscript. Z S and W W secured funding, critically revised the text and approved the finalized version.

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