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EDITORIAL COMMENT

Better Blood Flow Delivered

Extracellular Matrix-Derived Hydrogels for the Induction of Arteriogenesis in Peripheral Artery Disease?*

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Lower extremity peripheral artery disease (PAD) is a highly prevalent syndrome that affects approximately 10% to 20% of all individuals older than 65 years in the United States and Europe (1,2). Atherosclerosis is the dominant cause of PAD and frequently leads to a progressive and hemodynamically significant narrowing of lower extremity arteries that results in, for example, functional impairment, claudication, and critical limb ischemia (CLI), the latter being an important reason for limb amputations and disability in afflicted patients (3). Therefore, the medical management of PAD patients today mainly aims at: 1) slowing the progression of vascular disease by cardiovascular risk factor control; 2) reducing the increased risk of ischemic events caused by common comorbidities such as cerebrovascular and coronary artery disease; and 3) improving lower limb perfusion especially by interventional endovascular or open surgical revascularization treatment (3). However, not all PAD patients with CLI are eligible for revascularization or benefit from this intervention. Thus, for this subgroup of patients alternative strategies for the improvement of limb perfusion are needed to avoid progressive tissue damage.

Among the alternative strategies, therapeutic neovascularization has emerged as an attractive concept (4,5). The term *therapeutic neovascularization* refers to a controlled vascular expansion in tissues that have inadequate blood supply, via iatrogenic induction of angiogenesis (sprouting of new blood vessels from pre-existing ones, primarily resulting in an increased capillary density) or arteriogenesis, that is, the enlargement of pre-existing interconnecting arterioles leading to the formation of new collateral arteries (4,6,7). In this context, early studies have primarily focused on the therapeutic manipulation of the angiogenic process; however, results of clinical trials designed to substantiate the potential benefit of therapeutic angiogenesis in PAD or coronary artery disease patient cohorts have been disappointing (8). From these studies, it has become increasingly clear that arteriolar growth and maturation rather than sprouting angiogenesis may be key to effective neovascularization (4-7). Mechanistically, it has been speculated that the predominant induction of de novo capillary formation, as observed during treatment with pro-angiogenic molecules, primarily yields immature and hardly interconnected "high-resistance" capillary networks that are not able to restore tissue perfusion in the vascular beds of occluded arteries (5-7). Of note, stem cell-based salvage therapies in atherosclerotic vascular disease (that are under active clinical investigation in PAD patients) are hypothesized to act largely via the enhancement of angiogenesis, as these (heterogeneous) cellular therapeutics have been described to release considerable amounts of pro-angiogenic growth factors (9).

Considering the rather limited success of angiogenic interventions in PAD patients, therapeutic arteriogenesis may represent a more promising approach to normalize compromised arterial blood

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flow to ischemic tissues (4-7). Indeed, it has been demonstrated in the clinical setting that a well-developed collateral artery network due to efficient arteriogenesis in principle has the ability to compensate even for the complete occlusion of large arteries in humans (6,10). On the mechanistic level, arteriogenesis of the adult organism is believed to occur as a complex series of biomechanical as well as subsequent cellular signaling events that orchestrate a coordinated interaction of vascular as well as nonvascular cells to induce arteriolar growth. The key stimulus for arteriogenesis is an increase in blood flow and vascular endothelial shear stress due to the obstruction or occlusion of an artery (4-7). Fluid shear stress then triggers nitric oxide (NO) release from endothelial cells and NO-related vasodilation to further aggravate circumferential wall stress (4-7). Endothelial shear stress additionally induces a nuclear factor κ B-dependent induction of, for example, proinflammatory adhesion molecules (ICAM-1, VCAM-1), chemokines (MCP-1), and growth factors (PDGF-BB, angiopoietin-2) that attract monocytes to the vessel and stimulate smooth muscle cell proliferation and hypertrophy (4-7). Perivascular recruitment of monocytes is an important event in the arteriogenic process because these cells are a key source of vascular endothelial growth factor A (VEGF-A) and proteases, for example matrix metalloproteinase (MMP)-2, that are necessary to boost endothelial cell proliferation in the enlarging vessel via VEGF receptor (VEGF)-2 stimulation and to facilitate outward remodeling of the vessel through breakdown of the surrounding extracellular matrix (4-7). Finally, the self-limiting process ceases when fluid shear stress normalizes during progression of collateral vessel diameters.

Despite recent advances in the field of arteriogenesis, many details of this complex process have not been elucidated so far, and the list of suitable targets or interventional concepts for therapeutic manipulation of the arteriogenic process has remained short. In this inaugural issue of *JACC: Basic to Translational Science*, however, Ungerleider et al. (11) add a new attractive interventional concept to this list. In a set of elegant and thoroughly conducted in vitro and in vivo experiments, they establish a novel strategy to improve arterial blood flow and skeletal muscle remodeling in the rat model of hindlimb ischemia via direct delivery of extracellular matrix (ECM)-derived hydrogels to ischemic muscle tissue.

To achieve this goal, in a first step, decellularized ECM were prepared from porcine skeletal muscle tissue and human umbilical cords and further processed to yield a purified, pepsin-digested lyophilisate that, before its use, was converted to an isotonic injectable hydrogel by the addition of sterile water. A thorough characterization of these hydrogels involving, for example, global proteomics as well as scanning electron microscopy analyses performed upon gelation of these hydrogels in vivo revealed considerable differences in the molecular composition and mesh-like macrostructures of both matrices, that is, a more close-meshed and regular structure of the ECM-based hydrogel derived from porcine skeletal muscle tissue. Thus, the present study supports the current perception that ECM compositions considerably depend on the tissue studied and that biomaterials derived hereof, at least in part, retain the biomechanical and biochemical characteristics of their original tissue source (12,13).

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In a subsequent set of in vivo experiments, the impact of both hydrogel preparations on arterial blood flow recovery and blood vessel growth was analyzed in a rat hindlimb ischemia model after a single bolus injection of the biomaterial into the ischemic gracilis muscle 7 days after femoral artery ligation. In this experimental setup, perfusion of both ischemic and healthy limbs was regularly analyzed over a period of 6 weeks before and after the intervention. Most importantly, within 3 weeks after injection, both hydrogel preparations, to a similar extent, improved arterial blood flow to the ischemic muscle tissue as compared with control interventions, and this effect remained stable (or even further increased) during the remaining 2 weeks of the study. Additional morphometric analyses showed a higher density of large arterioles, but no change in the density of capillaries in the affected muscle tissue of hydrogel-treated animals. These findings led the authors to conclude that the (comparable) increase in blood flow induced by both hydrogels depended on the induction of arteriogenesis rather than angiogenesis, a conclusion that is supported by the existing literature in that: 1) development of a functional collateral circulation induces a significant shift towards an increase in arteriolar vessel size rather than an increase in arteriolar or capillary vessel density (6,10); and 2) arteriogenesis is the most prominent mechanism for blood flow recovery after femoral artery occlusion at least in small rodent models (6,10,14). However, the fact that similar arteriogenic effects were observed during treatment with the 2 hydrogels from different tissue sources indicates that a common (and still unknown) ECM-derived stimulus rather than tissue-specific ECM cues were necessary

to promote arteriogenesis in this model. In further histological analyses, the authors additionally focused on the ability of hydrogels to confer tissue (skeletal muscle) protection. Interestingly, in this setting skeletal muscle-derived hydrogels more strongly improved skeletal muscle progenitor recruitment and muscle architecture, thereby suggesting that skeletal muscle-derived ECM provides specific signals to foster skeletal muscle integrity during tissue ischemia.

Although the study contributes several valuable findings to the field of arteriogenesis research, it also has certain limitations. In this regard, one obvious limitation of the present study is that it cannot provide a conclusive explanation for its key observations, namely the proarteriogenic properties of acellular ECM-based hydrogels. In an attempt to fill the mechanistic gap, whole transcriptome data were captured in ischemic muscle tissue from hydrogel-treated and control mice 3 and 10 days after intervention. These analyses yielded 2 "hard-to-interpret" sets of transcriptome data that showed 561 differentially regulated genes early after intervention, whereas the number of differentially regulated genes 1 week hereafter dramatically decreased to 16, thereby indicating that the early response after hydrogel intervention may set the scene for an efficient arteriogenic response in this model. Interestingly, early after the intervention, a considerable up-regulation of several genes that have been described (15) to enhance perivascular monocyte recruitment during arteriogenesis, for example, Ccr2, Ccl5, and Cxcl10, along with an up-regulation of genes indicative of lymphocyte and smooth muscle cell proliferation, was observed. It is therefore tempting to speculate that the hydrogel components or its degradation products have the ability to enhance monocyte recruitment to sites of arteriolar growth. Upon recruitment, these cells then secrete, for example, VEGF-A, NO, and MMPs to stimulate vascular cell proliferation and the outward remodeling of arterioles. Nonetheless, future hypothesis-driven studies are needed to unravel hydrogel-related signaling pathways in skeletal muscle tissue that foster the arteriogenic process.

Despite these limitations, the study of Ungerleider et al. (11) goes beyond the state of the art in several ways and may additionally have important clinical implications. First, it discloses a previously unrecognized role of acellular ECM-based hydrogels in promoting arteriogenesis, blood flow recovery, and tissue protection in the setting of critical hindlimb ischemia in vivo. As acknowledged by the authors (11,16,17), several studies have explored the role of (less complex) biomaterials mostly as drug or cell delivery systems in PAD-related animal models. However, none of these studies has focused on the inherent potential of these biomaterials to induce blood flow and affect vascular remodeling on its own. Moreover, biomaterials and ECM-based scaffolds have been widely used in tissue protection and repair (also in the clinical setting) (13,16), but the relevance of tissue source for skeletal muscle protection conferred by ECM-based biomaterials in the context of ischemic vascular disease has not been examined so far. Second, this study paves the way for the development of tissue-specific hydrogel-related drug or cell delivery systems with superior tolerability profiles, delivery systems that could be additionally engineered to yield hydrogels with optimized biomolecular compositions and biomechanical properties. Finally, the study highlights a simple, yet effective technique to induce arteriogenesis in CLI that, in principle, allows for timely translation to the clinical setting; a similar myocardial ECM-based hydrogel designed by the same group has already reached the first phase of clinical development (16). Nevertheless, before translation of this technique to the clinic can take place, further information on, for example, efficacy, biocompatibility, and hemocompatibility, has to be captured from experiments in pre-clinical disease and large animal models.

Taken together, this valuable contribution of Ungerleider et al. (11) uncovers the ability of ECM-based hydrogels to improve arterial blood flow and skeletal muscle remodeling in a pre-clinical model of CLI via direct delivery of ECM-derived hydrogels to ischemic muscle tissue. Several questions, including the question of how the biomaterials exert their effects on arterial growth and remodeling, need to be answered in further hypothesis-driven animal studies that could also be used to obtain important safety information needed for further translational development of these potential biotherapeutics. However, as treatment options for patients with CLI remain scarce, induction of arterial blood flow to compromised tissues via delivery of ECM-based hydrogels may prove to be a valuable salvage strategy for these patients in the future.

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