Journal of Controlled Release 153 (2011) 217–224

Contents lists available at ScienceDirect

Journal of Controlled Release

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

First implantable device for hypoxia-mediated angiogenic induction

E. Hadjipanayi [a,1], U. Cheema [a,*,1], V. Mudera [a], D. Deng [b], W. Liu [b], R.A. Brown [a]

[a] UCL, Tissue Repair and Engineering Centre, Institute of Orthopaedics, Stanmore Campus, London, HA7 4LP, UK
[b] Department of Plastic and Reconstructive Surgery, Ninth People's Hospital and National Tissue Engineering Center of China, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, PR China

ARTICLE INFO

Article history:
Received 6 December 2010
Accepted 25 March 2011
Available online 31 March 2011

Keywords:
Nano-fibrillar collagen depot
Hypoxic pre-conditioning
Growth factor delivery
Angiogenesis
Vascularisation

ABSTRACT

Delayed or inadequate vascularisation is one of the major factors leading to tissue infarction and poor graft survival. Current vascularisation strategies that rely on delivering single growth factors have proved ineffective or hard to control in practise. An alternative approach has been identified by this group that relies on stimulation of physiological angiogenic factor cascades by engineering local cell-hypoxia, within a nano-fibrillar collagen material. Here we report on a novel, practical and effective implantable device for delivering engineered angiogenic signalling, on demand. Human dermal fibroblast-seeded dense-collagen depots were pre-conditioned under physiological cell-generated hypoxia to up-regulate production of key angiogenic factors, including HIF1α and $VEGF_{165}$. The level of $VEGF_{165}$ protein retained within depots (indicating general angiogenic factor production) was directly correlated to the duration of pre-conditioning. Angiogenic factor delivery from pre-conditioned, non-viable depots rapidly induced an angiogenic response within endothelial cell-seeded constructs *in vitro*, while implanted acellular 3D constructs incorporating such angiogenic depots in their core were infiltrated with perfused vessels by 1 week *in vivo*, at which stage non-angiogenic implants were minimally perfused. Depot stability, tuneability of cell/matrix composition with long clinical experience of the collagen material, together with cost effectiveness, make this angiogenic therapy a promising addition to a clinician's tool kit for improving local tissue perfusion.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Promoting the growth of new blood vessels in ischaemic diseases, such as myocardial infarction, stroke or peripheral vascular disease represents an appealing approach for treating these devastating conditions. Furthermore, successful use of grafts and engineered implants to aid tissue repair/regeneration critically relies on their rapid vascularisation for optimal survival and integration within the host [1]. Consequently, the ability to control the timing, location and direction of engineered angiogenesis has become a therapeutic holy grail. Strategies to date have focused mainly on stimulating angiogenesis by delivery of commonly one or two pro-angiogenic factors (at the gene [2,3] or protein levels [4–7]), cell-based therapies [8–10], or combinations of each [11–13].

Despite successful induction of revascularization of ischemic tissues upon local delivery of single angiogenic growth factors in animal models, similar efforts have shown only modest benefit in human clinical trials [9,14]. Possible reasons for this relate to inefficient delivery of the angiogenic proteins (e.g. their short half-life *in vivo*), the risk of adverse events due to unbalanced dosage (e.g. hypotension, vascular leakage and tumour formation), and incomplete understanding of which growth factor combinations and sequences produce effective angiogenesis [9,14,15].

Ischaemia-induced angiogenesis is a physiological response to tissue hypoxia, orchestrated by the transcriptional activator hypoxia-inducible factor 1α (HIF-1α) [16]. HIF-1α stabilisation induces, directly or indirectly, a plethora of angiogenic mediators such as vascular endothelial growth factor (VEGF), platelet-derived growth factor B (PDGFB), placental growth factor (PLGF), angiopoietins 1 and 2, and matrix metalloproteinases [17]. Given that the formation of a functional, mature, and durable vascular network is complex, the ability of this master regulator to induce several mediators of angiogenesis prompted the concept that strategies designed to increase HIF-1α activity (e.g. by pharmacological stabilisation or over-expression through gene transfer) might be more efficient in inducing angiogenesis/arteriogenesis after ischemic events (e.g. hind limb, cardiac or cerebral ischemia) than those relying on single factors [9,18–22]. Indeed, the importance of switching-on hypoxia-induced angiogenesis at the onset of the process is increasingly appreciated [22,23].

In contrast to exogenous delivery of angiogenic factors, cell-generated angiogenic cascades commonly result in a more functional vasculature [1,24,25]. The efficacy of transplanting autologous bone-marrow stromal cells, mesenchymal stem cells and endothelial progenitor cells to treat patients with ischemic heart disease and

* Corresponding author at: UCL Division of Surgery & Interventional Science, Institute of Orthopaedics and Musculoskeletal Science, Brockley Hill, Stanmore, HA7 4LP, UK. Tel.: +44 20 8909 5888(direct); fax: +44 20 8954 8560.
E-mail address: u.cheema@ucl.ac.uk (U. Cheema).
[1] Both authors have contributed equally to this work.

0168-3659/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2011.03.029

ischemic limbs is currently being tested on a large scale [1,9]. This is because well-orchestrated production of angiogenic factors, in terms of combinations, sequences and concentrations, is presently only possible through the use of whole cell systems, releasing a host of angiogenic proteins under native feedback regulation. Therefore, a strategy that focuses on harnessing the natural mechanism that promotes angiogenesis in the body, i.e. physiological tissue hypoxia, while incorporating this into controlled biomimetic analogues of extracellular matrices, could not only succeed in induction of physiological angiogenesis, but also control its onset, location and direction.

Culturing cells under physiological hypoxia (1–10% O_2/pO_2 of 7.6–76 mmHg) is currently employed as a strategy to control cell behaviour, in particular up-regulation of angiogenic signalling molecules [26–30]. For example, hypoxic pre-conditioning of implanted bone marrow stem cells has been shown to increase their angiogenic potency through VEGF up-regulation [31,32]. We previously demonstrated that it is possible to control the local O_2 microenvironment within 3D collagen constructs by adjusting the seeding cell density and spatial position, therefore total cell-depot O_2 consumption [33,34]. Seeding constructs with human dermal fibroblasts (HDFs) at high density resulted in rapid reduction of core O_2 tension towards the low end of the physiological hypoxic range, which elicited a multifold up-regulation of VEGF gene expression [34]. Up-regulating hypoxia-induced signalling (*HIS*) by engineering local cell-hypoxia, then, is an important strategy for controlling physiological angiogenesis *in vitro* and *in vivo* [35]. The present study tests the concept of using the simple end of this spectrum, namely the angiogenic effectiveness of delivering *HIS* angiogenic factors, trapped within a preserved, collagen-material depot, without living cells.

Choosing appropriate vehicles for delivering angiogenic factors to clinically required locations is at least as important as the choice of factor source (e.g., genes, recombinant proteins or cell-produced factors), since the vehicle's material properties (e.g. porosity, pore size, degradation rate, factor binding affinity) critically determine the factor release kinetics [14,36]. Furthermore, in the case of cell-generated factors, the delivery vehicle must have the capacity to support the cell population, while retaining secreted proteins [37]. Therefore, the ability to precisely engineer a biomimetic depot biomaterial, with its cell population, is essential. Previous work from this group has developed a fabrication platform for biomimetic engineering of collagen [38], retaining the advantages of collagen materials (i.e., biocompatibility, low immunogenicity and ability to undergo natural cellular remodelling). Plastic compression (PC) of collagen hydrogels rapidly produces natural cellular materials with controllable cell/matrix density, tissue-like nano-/meso-scale architecture, mechanical properties and biomimetic function [38].

Here we test the hypothesis that angiogenic factors ($VEGF_{165}$ as exemplar), produced by pre-conditioning dermal fibroblasts to cell-generated physiological hypoxia within dense-collagen scaffolds, will be retained by the collagen material after freeze/thaw cell killing, to produce non-viable angiogenic depots (Fig. 1). Depot angiogenic effectiveness was tested *in vitro* by embedding them into human umbilical vein endothelial cell (HUVEC)-seeded constructs, while their ability to induce directional angiogenesis was tested *in vivo* by implanting 3D acellular collagen constructs, incorporating depots in their core, subcutaneously in rabbits. Depot angiogenic factor content was controlled by varying the duration of hypoxic pre-conditioning and the seeding cell density.

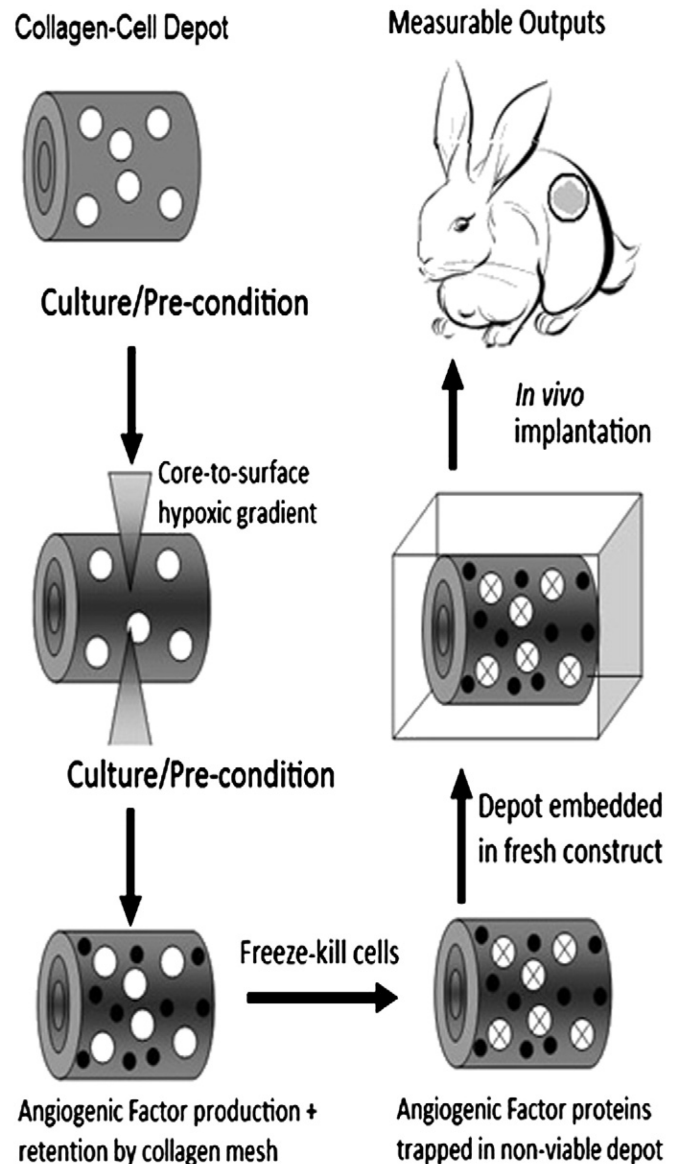

Fig. 1. Schematic of concept for development of an implantable device to deliver hypoxia-induced angiogenic signalling on demand. A dense-collagen cellular depot (of specified cell/material density) is pre-conditioned *in vitro* for a desired period. Cellular O_2 consumption generates a core-to-surface hypoxic gradient within the depot, whose level and duration can be controlled by adjusting the seeding cell density and length of culture [34]. Exposure of cells to physiological hypoxia up-regulates production of angiogenic factor proteins which are retained by the collagen material (nano-porous matrix) and remain trapped within the depot after the seeded cells are killed by snap-freezing. Non-viable depots are then embedded into fresh constructs which are implanted *in vivo*. Depot functionality (i.e. release of trapped angiogenic factor proteins) can be assessed by measuring capillary in-growth into the implant, relative to non-hypoxic baseline depots.

2. Materials and methods

2.1. Cell culture

Adult human dermal fibroblasts (HDFs) and male New Zealand white rabbit dermal fibroblasts (RDFs) were cultivated in DMEM supplemented with 10% FCS (First Link, UK), 1000 U/ml penicillin and 100 mg/ml streptomycin (Gibco, UK). Human umbilical vein endothelial cells (HUVECs) were cultured in complete endothelial cell growth medium (Promo Cell, Germany).

2.2. Scaffold fabrication and culture

2.2.1. Construct plastic compression

Acellular, HDF-, HUVEC- and RDF-seeded rat-tail type I collagen gels (5 ml) were prepared as previously described [38]. Acellular collagen gels and gels seeded with 2×10^6 HDFs, 5×10^5 RDFs or 2×10^6 RDFs were cast in rectangular moulds (size: $4.5\times1.5\times1$ cm). Following 30 min setting gels were compacted by plastic compression to produce ~200 μm thick sheets (Fig. 2a), which does not significantly reduce cell viability for HDFs, HUVECs, or human bone marrow derived stem cells

Download English Version:

https://daneshyari.com/en/article/1424976

Download Persian Version:

https://daneshyari.com/article/1424976

Daneshyari.com