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Review Article

Growth Factor Delivery for Tissue Engineering

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A tissue-engineered implant is a biologic-biomaterial combination in which some component of tissue has been combined with a biomaterial to create a device for the restoration or modification of tissue or organ function. Specific growth factors, released from a delivery device or from co-transplanted cells, would aid in the induction of host paraenchymal cell infiltration and improve engraftment of co-delivered cells for more efficient tissue regeneration or ameliorate disease states. The characteristic properties of growth factors are described to provide a biological basis for their use in tissue engineered devices. The principles of polymeric device development for therapeutic growth factor delivery in the context of tissue engineering are outlined. A review of experimental evidence illustrates examples of growth factor delivery from devices such as micropaticles, scaffolds, and encapsulated cells, for their use in the application areas of musculoskeletal tissue, neural tissue, and hepatic tissue.

KEY WORDS: tissue engineering; growth factors; controlled release; bone; nerve; liver.

INTRODUCTION

Growth Factors

Growth factors are polypeptides that transmit signals to modulate cellular activities. Growth factors can either stimulate or inhibit cellular proliferation, differentiation, migration, adhesion and gene expression. There are several characteristic properties of growth factors. Many cell types can produce the same growth factor and the same growth factor can act on many cell types (pleitropism) with the same or different effects. Furthermore, different growth factors can share the same biological effect (redundancy). Growth factor effects are concentration-dependent, often in a complex non-monotonic way. Growth factors can influence the secretion and action of other growth factors (antagonize or enhance). Growth factors are not stored as preformed molecules but their secretion is a brief self-limited event and their synthesis is initiated by new gene transcription, transient transcriptional activation, and their mRNAs are unstable. There is transient synthesis, rapid release with activity controlled by post-transcriptional mechanisms such as proteolytic release of an active product from an inactive precursor. Most cellular responses to growth factors require new mRNA and protein synthesis.

Growth factors initiate their action by binding to specific receptors on the surface of target cells. Depending on the proximity of their site of synthesis to their site of action, growth factors have been classified as endocrine (target cell is distant), paracrine (target cell is nearby), autocrine (target cell is the same cell that secreted the growth factor), juxtracrine (target cell is apposed to growth factor/receptor complex) or intracrine (growth factor/receptor complex is internalized). Hundreds of growth factors have been identified, characterized and, based on structural homologies, grouped into at least twenty families and super-families (1,2,3).

Growth factors usually exist as inactive or partially active precursors that require proteolytic activation, and may need to bind to matrix molecules for activity or stabilization. Growth factors have a short biological half-lives. Platelet-derived growth factor (PDGF), isolated from platelets, can not be detected in the circulation and has a half life of less than 2 minutes when injected intravenously (4).

The availability of growth factors from the conditioned medium of cultured human cells, their expansion through recombinant technologies, and improved understanding of their functions and clinical applications has increased the need for pharmaceutical forms. Unfortunately, the short half-lives of growth factors, their relatively large size, slow tissue penetration, their potential toxicity at systemic levels all leading to a long time for tissue to respond, obviates conventional routes of administration.

Polymeric Devices for Therapeutic Growth Factor Delivery

One way of enhancing the *in vivo* efficacy of growth factors is to facilitate the sustained release of bioactive molecules over an extended time period by their incorporation into a polymer carrier. Implantation of a drug delivery device directly into the tissue in need of treatment facilitates localized drug delivery. Delivery systems have been designed in a variety of geometries

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and configurations (reservoirs, matrices) and have been fabricated from diverse types of natural and synthetic polymers (degradable, non-degradable). These devices have a common ability to control the release of bioactive proteins for extended periods of time, but by different mechanisms. Through incorporation into polymeric devices, protein structure and thus biological activity can be stabilized, prolonging the length of time over which activity is released at the delivery site. The duration of drug release from a polymer matrix can be regulated by the drug loading, type of polymer used, and the processing conditions. Adverse processing conditions that cause protein aggregation or denaturation need to be avoided. Maintenance of protein stability in devices upon exposure to moisture needs to be improved. Recent reviews describe materials used for protein delivery systems (5,6) and the mechanisms of release (6,7).

In tissue engineered devices, there are two different potential delivery systems. Growth factors can be incorporated directly into the scaffold at (8,9) or after fabrication (10,11). In a biodegradable system, the growth factor would be released as the scaffold degrades to induce tissue regeneration. Growth factor, directly incorporated into a bioresorbable polymer scaffold, is released by a diffusion-controlled mechanism that is regulated by the median pore size such that different pore sizes affect the tortuosity of the scaffold and thereby control the release of the protein (9,12). The protein can also be released by an erosion mechansim or a combination with diffusion. Alternatively, the growth factor delivery device, in the form of microparticles, nanoparticles, fibers or injectable complexes, can be incorporated into the scaffold (13). The growth factor release rate would be dependent on rate of degradation of the delivery device, with some contribution from that of the scaffold material, and rate of growth factor diffusion through pores of the delivery device and of the scaffold. For each tissue-engineered device and application, the growth type(s), dosage, release pattern (constant, pulsatile, and time programmed), kinetics of release and duration of delivery should be optimized. These parameters need to be optimized for each growth factor delivered. Successful delivery of growth factor(s) requires targeting responsive cells, at the required pharmacological concentration while maintaining the stability of the active form of the growth factor(s). Cell responsiveness is determined by their level of growth factor receptor expression and would clearly determine the effectiveness of an appropriately delivered factor.

Specific growth factors, released from the delivery device, would aid in the induction of host parenchymal cell migration, proliferation and differentiation or improve engraftment of seeded cells, supported by the polymer scaffold, for more efficient tissue regeneration. The three-dimensional polymer scaffold defines the volume of the newly formed tissue. There should be no interference of the growth factor delivery device and the tissue engineered device. The two components should function synergistically. Growth factors released from a device may interact with matrix proteins in the scaffold or in the surrounding tissue to enhance their local bioavailability or provide increased stability. The host response to the carrier material should not be detrimental, due to extensive fibrosis, inflammatory or immune responses, to the effectiveness of growth factor delivery from the device to the surrounding tissue (14).

Another way to deliver growth factors is via co-transplantation of growth factor-secreting natural or genetically engineered cells, sometimes along with the tissue forming cells, within the device (15). Growth factors, immobilized on a biomaterial surface are able to control cell proliferation (16,17,18) and co-immobilized adhesion factors mediate cellular adhesion (16). Such materials are able to regulate tissue formation with artificial biomaterials.

Tissue Engineered Implants

A tissue engineering implant is a biologic-biomaterial combination in which some component of tissue has been combined with a biomaterial to create a device for the restoration or modification of tissue or organ function (19). There are several types of devices that are important in tissue engineering (20). Polymer matrices are used to control and guide wound healing and tissue regeneration, to elicit specific cellular interactions, functions and tissue responses and to serve as scaffolds to support cell transplantation. Specific control of tissue regeneration is achieved by controlled growth factor/cytokine release from devices or transplanted cells. Immobilized bioactive ligands on biomaterials (biomimetic materials) control single and multiple cellular morphology and function via receptor-mediated processes. Biomaterial barriers block molecular signals that stimulate scar formation and immune rejection.

This review will focus on the second strategy for growth factor delivery for tissue engineering. Particular illustrative examples will be given in which growth factors are delivered from a tissue-engineered device to facilitate tissue repair and restoration of organ function. Three application areas, musculoskeletal tissue, neural tissue, and hepatic tissue, represent major thrust areas in tissue engineering and growth factor delivery. Readers are referred to other reviews of growth factor delivery for tissue engineering (21) with descriptions of other application areas such as the vascular system (2).

TISSUE ENGINEERING APPLICATION EXAMPLES

Musculoskeletal Tissue

Tissue induction is the process by which ingrowth of tissue adjacent to a biomaterial is effected. For bone induction, osteoconductive biodegradable materials repair bone defects by relying on bone replacement by creeping substitution of osteoblasts from the borders of the defect. Osteoinduction for healing of otherwise non-union defects, can be caused by the action of various factors such as pre-seeded osteoblasts and/or bioactive molecules incorporated into a polymer scaffold or supplied by a stabilized hematoma (22). The most common osteogenic factors are the members of the transforming growth factor-β (TGF-β) superfamily of which TGF-β1, bone morphogenetic protein-2 (BMP-2), and osteogenic protein (OP-1 or BMP-7) are members (3).

Because the event initiating osteogenesis involves a pleiotropic protein acting on a pluripotent cell (23) new bone will not necessarily form (24). In fact, recombinant human bone morphogenetic protein-2 (rhBMP-2) differentially induced pluripotent mesenchymal stem cell differentiation depending on the concentration applied *in vitro*: low concentrations favored adipocytes and high concentrations chondrocytes and osteoblasts (25). Furthermore, the context in which rhBMP-2 was

presented to the pluripotent mesenchymal stem cell could be controlled by extracellular matrix proteins to induce the desired cell type without the dose dependency constraints. Dose-dependent effects have been noted for OP-1 as well (26). The local growth factor concentration is clearly one means of inducing the desired osteoblast differentiation while simultaneously discouraging development of competing fibroblast, adipocyte, chondroblast phenotypes in a tissue engineered device. Other device characteristics that will determine the type of tissue induced include porous microarchitecture of the scaffold (22), surface properties (27), and extent of early vascularization (28).

Various carriers for osteogenic proteins have been developed and are presented in Table 1. Some of these have shown evidence of ectopic bone formation. Protein pharmacokinetics were found to be dependent on the carrier type (29). Retention of rhBMP-2 was variable among different carriers after 3 hours (range 10–75%). Collagenous sponges retained the highest fraction of the implanted dose. There was a gradual loss of rhBMP-2 with kinetics strongly dependent on the implanted carrier. Collagenous carriers lost rhBMP-2 gradually from the implant site while mineral-based carriers such as synthetic and bovine-derived hydroxyapatite particles retained a comparatively higher fraction of the implanted rhBMP-2. In another study, rapid diffusion and clearance of the growth factor from the implant site was overcome by covalently binding TGF-β2 to injectable bovine dermal fibrillar collagen via difuctional polyethylene glycol, with concomitant increases in growth factor stability and local bioactivity (30).

Several protein/carrier combinations have demonstrated regeneration of bone in structural defects. The following examples are grouped according to the material used, natural, synthetic, or a composite of both, and the growth factor delivered.

Collagenous materials are the most common natural materials for growth factor delivery for bone regeneration. Crosslinked gelatin hydrogels, with bFGF incorporated via electrostatic interaction (31), implanted into rabbit cranial defects, enhanced bone regeneration with defect closure after 12 weeks as compared to free bFGF of the same dose without carrier (32). The slower the degradation rate of the hydrogel as determined by a lower water content, the higher the extent of bone regeneration; retention of osteoblasts was enhanced. These crosslinked gelatin hydrogels have also been used to deliver rhBMP-2 to rabbit cranial defects with bone formation, but to the same extent as free rhBMP-2 of the same dose in this site (33). Helistat® C, a crosslinked atelopeptide derived from bovine type I collagen, used as a scaffold for the delivery of rhBMP-2, demonstrated regeneration of osseous contour by 8 weeks in unilateral critical sized defects in the radii of rabbits (34). Helistat® delivering rhBMP-2 was as effective as the gold standard autograft in new bone formation. Untreated defects and those with Helistat® alone showed little new bone formation.

Synthetic polymer scaffolds for cell seeding, growth factor delivery and bone regeneration have focused primarily on the poly(α-hydroxy acid) family of polymers. Unilateral critical sized defects in rabbit radii, treated with a scaffold of poly(D,L-lactic acid) (PDLLA) delivering rhBMP-2, demonstrated greater radiopacity (equivalent to the autograft treatment) as well as greater torque at failure as compared to untreated controls (35). Platelet-derived growth factor-BB (PDGF-BB), incorporated into poly(L-lactic acid) (PLLA)-coated on poly(glycolic acid) (PGA) meshes, increased new bone formation in rat calverial defects and completed bony reunion after 2 weeks of implantation (36). The pores of a PDLLA mesh, of a macrostructure optimized to the architecture of cancellous bone, were coated with a filamentous velour of hyaluronan. RhBMP-2 was co-solubilized with hyaluronan for its delivery to facilitate bone regeneration in a critical sized defect in rabbit radii, and in a canine inter-transverse process spinal fusion (37).

Microparticles of gelatin and poly(α-hydroxy acid) have been investigated for growth factor delivery to facilitate bone regeneration. Different carrier systems, including collagen sponges and bioabsorbable poly(D,L-lactic-*co*-glycolic acid)

Table 1. Carriers for Osteogenic Growth Factors

Growth Factor	Carrier	Reference
BMP-2	Poly(α-hydroxy acids)	(29, 78)
	Poly(DL-lactic-co-glycolic acid) (PDLLGA)/gelatin microcapsules	(79)
	Hydroxyapatite porous particles and coral-replicated porous tablets	(80)
	Synthetic and bovine-derived hydroxyapatite particles, and coral-derived hydroxyapatite	(29)
	Gelatin capsules loaded with PDLLGA microparticles and demineralized freeze-dried bone allografts	(81)
	Si-Ca-P porous glass (xerogels)	(82)
	Chemically crosslinked absorbable collagen, dehydrothermally crosslinked collagen sponge	(29)
	Absorbable gelatin sponge	(29, 83)
	Glutaraldehyde cross-linked gelatin	(84)
	Tricalcium phosphate	(29)
	Rat demineralized bone matrix, and delipidated bovine bone matrix	(29)
	Human demineralized bone matrix, thermoashed bone mineral, nondimeralized bone particles, and irradiated cancellous chips	(29)
OP-1 (BMP-7)	Polyphosphate	(85)
TGF-β1	PDLLGA microparticles	(86, 87)
	PDLLGA/demineralized bone matrix rods	(88)
	Si-Ca-P porous glass (xerogels)	(89)
	Ethylene-vinyl acetate (EVA) copolymer rods for delivery of platelet-derived growth factor (PDGF-BB) and/or TGF-β1	(90)

(PDLLGA) particles stabilized in an allogeneic blood clot, for rhBMP-2 delivery, induced osteogenesis within a region defined by osteopromotive expanded polytetrafluoroethylene (ePTFE) membranes in transosseous rat mandibular defects (38). For bone formation under osteopromotive membranes, the PDLLGA carrier appeared superior to collagen for rhBMP-2 delivery: both were more efficient in bone formation than membranes without BMP and carrier. The presence of osteopromotive membranes, to define the region for bone growth, prevented lateral bone formation. Femoral defects in rats, treated with small diameter PDLLGA microparticles and rhBMP-2, stabilized in a blood clot, showed a dose dependent increase in failure torque and a higher incidence of union, the highest dose showing the greatest effect (39). RhBMP-2, mixed with PDLLGA microparticles and stabilized in an autologous blood clot, hydroxypropyl methylcellulose or sodium alginate cross-linked with calcium ion, implanted into rat calvarial defects, demonstrated more new bone formation than the controls (carrier, no rhBMP-2) (40).

The ceramic materials of β-tricalcium phosphate-monocalcium phosphate monohydrate (β-TCP-MCPM) cement cylinders loaded with rhBMP-2 used for the treatment of rat femoral critical-size defects showed a dose dependent incidence of union at 3 weeks (41). No instances of union were observed in the defects implanted with cylinders only. PDGF-loaded hydroxyapatite rods, implanted into the medullary canals of rabbit femora, appeared to exhibit more bone in-growth than did those without PDGF, but not significantly (42). A bone wax polymer composed of 60 weight percent β-TCP and 40 weight percent poly(ϵ-caprolactone-*co*-glycolide) containing TGF-β1 was tested to treat standard-sized rabbit calvarial defects (43). However, incomplete biodegradation of the polymer ceramic composite retarded bone regeneration.

In a recent study, radiomorphometric assessment of athymic rat calvaria critical-sized defects at 2 and 4 weeks revealed that a composite of porous PDLLA with gelled type I collagen (PLC) containing rhBMP-2 (PLC/rhBMP-2) or rhBMP-2 and osteoblast precusor cells (OPCs) (PLC/OPCs/rhBMP-2) showed more radiopacity than either PLC or PLC/OPCs without rhBMP-2 (44). By 4 weeks, PLC/OPCs/rhBMP-2 and PLC/rhBMP-2 had regenerated the defects with more new bone than the other treatments. The authors described developing a system in which OPCs would be genetically engineered to constitutively express BMP to be delivered in a scaffold such as PLC.

Neural Tissue

Improved understanding of the mechanisms of neurological diseases opens the possibility for replacement therapy and regeneration. The presence of the blood-brain barrier complicates the passage of systemically delivered therapeutic molecules (45). Direct delivery to the nervous system via drug delivery systems (46) and transplantation of cells with (47) and without encapsulation (48) are some of the methodologies that are being developed. A combination of cell transplantation and neurotrophic and growth factor delivery may be the optimal for the treatment of neurodegenerative diseases (49), which are characterized by nerve cell loss, and neuronal regeneration (50).

Macrocapsules, prepared by filling preformed poly(acryonitrile-*co*-vinyl chloride) hollow fiber tubular structures with cells, have been used as continuous source of growth factors for the treatment of neurodegenerative diseases. The encapsulated cells provide a continuous source of neurotrophic factors as long as the cells remain viable with stable transgene expression. The physical barrier of the permselective polymer membrane prevents contact with certain immune components of the host facilitating transplantation of allogeneic and possibly even xenogeneic cells into the central nervous system without immune suppression. Baby hamster kidney (BHK) cells were genetically modified to secrete human nerve growth factor (NGF) for the treatment of Alzheimer's disease (51) and Huntington's disease (52). Furthermore, BHK cells have been genetically modified to secrete glial cell line-derived neurotropic factor (GDNF) (53) for the treatment of Parkinson's disease and to secrete human ciliary neurotrophic factor (CNTF) for the treatment of ALS (54) with restorative effects on neuron function.

Nerve growth factor delivered from encapsulated genetically modified BHK cells promoted the survival of axotomized septal cholinergic neurons in the rat brain (55). The delivery of GDNF from genetically-modified encapsulated cells sustained the function and survival of grafted embryonic dopaminergic neurons in the adult rat striatum (49). In a model of motoneuron death induced by siatic nerve section in newborn rats, brain-derived neurotrophic factor (BDNF) and GDNF, administered simultaneously but by distinct routes [single injection, continuous delivery from a controlled release device or encapsulated cells or repeated systemic injections (BDNF)], potentiated injured neuronal survival (56). Furthermore, even continuous delivery of each of these trophic factors alone was not able to completely abrogate the time-dependent decline in their rescue effects in this model of motoneuron death.

Synthetic nerve guidance channels of ethylene-vinyl acetate that released incorporated basic fibroblast growth factor (b-FGF) facilitated regeneration of cables bridging both nerve stumps, which contained nerve fascicles with myelinated and unmyelinated axons and their presumptive Schwann cells (57). Channels releasing both b-FGF and α_1-glycoprotein containined more myelinated axons than those releasing only b-FGF. The authors explained the effect of b-FGF as either a direct action of b-FGF on the neural elements or an indirect action of by stimulating Schwann cell proliferation. Transplanted Schwann cells within semipermeable poly(acryonitrile-*co*-vinyl chloride) polymer tubes, used as guides for peripheral nerve regeneration, stimulated more regeneration than the same tubes without Schwann cells in rats (58). However, in this model, propriospinal but not supraspinal axons grew into the channel. Indeed, infusion of BDNF and neurotrophin-3 (NT-3) enhanced propriospinal axonal regeneration and significantly promoted regeneration of specific distant populations of brain stem neurons into grafts at the mid-thoracic level in the adult rat spinal cord (59). Presumably, cells genetically-engineered to supply these same neurotrophic factors could also be co-transplanted with the Schwann cells. Neurotropic growth factor delivery and cell transplantation could potentially be combined with other synthetic biodegradable scaffolds made of the poly(α-hydroxy acid) family of polymers (60,61) and polyphosphazenes (62) and of natural scaffolds such as collagen-glycosaminoglycan (63), laminin-fibronectin double coated collagen (64) and hyaluronic acid (65) used in nerve regeneration.

Controlled-release systems for neurotrophic protein delivery have been developed from polymer matrices such as poly(-ethylene-*co*-vinyl acetate) (66) and microparticles of poly(D,L-lactic-*co*-glycolic acid) and poly (ε-caprolactone) (67). When proteins are directly delivered to brain tissue from a polymer, their concentration in the surrounding tissue decreases exponentially with distance away from the polymer, suggesting a tissue transport mechanism of diffusion and consumption (68). In order to modify the proteins such that they are more slowly consumed by the surrounding tissue, dextran conjugation of NGF has been successful at improving penetration and retention in the brain (69). Conjugation of poly(ethylene glycol) 2000 with murine NGF (mNGF) is another means of enhancing protein stability and therefore, effectiveness (70). This PEG-2000-mNGF was biologically active and exhibited reduced immunorecognition capability by specific antibodies. Presumably, the effects of such protein modifications would enhance the bioactivity of the delivered protein.

Hepatic Tissue

Hepatic tissue engineering is a means of supporting or replacing diseased liver function. Hepatocytes, transplanted within a synthetic degradable polymer must remain viable in sufficient numbers, with retention of their differentiated phenotype. While several studies have demonstrated hepatocyte engraftment and survival on synthetic porous biodegradable polymer scaffolds following heterotopic transplantation (71,72), long term maintenance and hepatocyte proliferation has not been achieved. The inability to mimic normal liver growth and regeneration is a limiting factor.

The molecular signals that regulate hepatocyte proliferation and function in tissue engineering are being investigated. Poly(D,L-lactic-*co*-glycolic acid) microparticles containing epidermal growth factor (EGF) co-transplanted with hepatocytes in poly-(vinyl alcohol)-coated poly(L-lactic acid) [PVA-coated PLLA] scaffolds enhanced engraftment of hepatocytes (13). This effect was only observed if a suitable environment was provided to the transplanted cells with delivery of hepatotropic factors from the portal circulation via an end-to-side portacaval shunt. Known hepatotropic factors include epidermal growth factor (EGF), acidic-fibroblastic growth factor (a-FGF), hepatocyte growth factor/scatter factor (HGF/SF), and transforming growth factor-α (TGF-α) (73). However, the precise combination of hepatotropic factors in the portal circulation is unknown.

Hepatocytes isolated from HGF/SF transgenic mice survived transplantation within PVA-coated PLLA scaffolds into wild type mice better than did similar transplantation of wild type hepatocytes (74). Presumably, the transgenic hepatocytes secreted HGF/SF with autocrine effects on cell survival. This study also indicated that genetically-modified animals can be used as a source of cells with growth factor-releasing capabilities. However, in this study, at time points up to 4 weeks, significant inflammatory reactions appeared to mitigate the positive growth factor effects. The authors concluded that the ability to control the inflammatory response as well as improve angiogenesis would further enhance hepatocyte survival. In other studies, co-transplantation of islets has improved the survival of transplantated hepatocytes (75,76). Adequate vascularization of the hepatocyte transplantation site does in fact appear to be important, as exogenous growth factor-induced prevascularization of the implant site with delivery of endothelial cell growth factor (ECGF) from chitosan-albumin (CA) microspheres enhances hepatocyte survival following their transplantation subcutaneously on CA scaffolds (77).

The growth and liver-specific phenotype of hepatocytes is regulated by a variety of soluble signals (e.g., growth factors and hormones), extracellular matrix and cell-cell adhesive signals. Integrated understanding and design of a tissue engineered liver incorporating both soluble and adhesive signals is expected to yield devices with the most physiological liver regeneration and function.

FUTURE CONSIDERATIONS

Animal experiments are beginning to demonstrate that growth factors delivered from a device can influence the regeneration of damaged tissues, enhance the engraftment and function of co-delivered cells, and ameliorate disease states. In the future, there will be advances in the understanding of the molecular basis of disease, of the molecules involved in tissue regeneration and of the local physiology surrounding tissue engineered constructs. These advances will assist in more precisely defining which growth factors need to be delivered, during what period of time and at which concentrations. These advances will certainly improve experimental results and may lead to clinical application. Once the inter-relationships between different growth factors are characterized and defined, carriers will need to be developed that can control the delivery of multiple growth factors in an orchestrated fashion. It is also important to characterize the cells in the implant mileau, since their response to delivered factors is largely defined by their specific receptor expression level. In the future, there will be further development of intelligent biomaterials with which the host interacts physiologically and that can respond appropriately to the implant location to elicit a desired morphogenetic response from the host based on geometry and chemical nature of the material. Such materials can be optimally developed for the delivery of one or more growth factors. The availability of new delivery systems may be essential for testing hypotheses regarding the relationship between therapeutic effect and release rate.

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