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Growth factor delivery from self-assembling nanofibers to facilitate islet transplantation

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Abstract

Recent advances in nanotechnology and molecular self-assembly may provide novel solutions to current cell transplantation deficiencies. Heparin-binding peptide amphiphiles (HBPAs) self-assemble from aqueous media into nanofibers that bind growth factors through interactions with the bioactive polymer heparin. In this report, we demonstrate that delivery of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) from HBPA scaffolds significantly increases blood vessel density in the mouse omentum over control scaffolds without growth factors ($P<0.0005$) and significantly enhances islet engraftment. Diabetic recipients transplanted with 250 isologous islets and HBPA scaffolds containing VEGF/FGF-2 achieved normoglycemia at a higher rate (78%) than control animals receiving identical scaffolds without growth factors (30%; $P<0.05$) or growth factors alone (20%). These data indicate that the enhanced engraftment can be attributed to specific growth factor effects that were made possible by the delivery mechanism of HBPA nanostructures.

Keywords

islet transplantation; omentum; peptide amphiphile nanofibers; fibroblast growth factor-2; vascular endothelial growth factor

Clinical islet transplantation trials have resulted in promising therapeutic benefits for select patients with type 1 diabetes(1,2). Despite many advances, the procedure's clinical potential remains limited by suboptimal engraftment and lingering concerns about the intrahepatic transplant site(3–6). Recent advances in nanotechnology and molecular self-assembly may provide novel solutions to address these issues. We recently developed a specific family of molecules known as peptide amphiphiles (PAs) that self-assemble from aqueous media into

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gels consisting of nanofibers that can be customized to present biological signals to cells in high density(7–9). By presenting appropriate biological signals, PA nanofibers have the potential to create more favorable environments for transplanted islets and facilitate functional engraftment in extrahepatic locations that have been previously plagued by failure.

In this study, we used heparin-binding PAs (HBPAs)(10) to deliver angiogenic growth factors to extrahepatic islet isografts in diabetic mice. Heparin-binding PAs (HBPAs) form nanofibers that bind strongly to heparin and related glycosaminoglycans(10). These matrix components play critical roles in growth factor binding, storage, and activation(11). We used the heparin-binding nanostructures to attach vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), two growth factors which are known to play critical roles in islet revascularization, survival, and function(12–15). Attachment to the HBPA nanostructures via heparin is intended to protect the growth factors from proteolysis and activate them for signaling through exposure of their receptor binding domains(10). The effectiveness of the HBPA delivery strategy is confirmed by recent work showing extended growth factor release *in vitro* and significant angiogenesis *in vivo* using a rat cornea model(10).

The purpose of this model study was twofold: **(a)** to assess the bioactivity of VEGF/FGF-2-binding HBPA scaffolds implanted in the mouse omentum, and **(b)** to evaluate the impact of VEGF/FGF-2-binding HBPA scaffolds in a functional islet isograft model. The experimental design incorporated 46 transplant recipients and six control groups, as outlined in Figure 3a. Diabetes was induced in 8–12 week male FVB/N mice (25.5–30 g) with intraperitoneal streptozotocin and islets were isolated from adult male FVB/N strain mice (Jackson Laboratories) as previously described(16). Islets were quantified by hand-picking in a double-blinded manner and each islet preparation (16 total) was distributed over multiple experimental groups during transplantation.

Scaffolds consisting of VEGF/FGF-2-binding HBPA gels in fibrous poly(L-lactic acid) (PLLA) matrices were prepared as follows. For each recipient, 7 μL of aqueous HBPA (30 mg/mL; synthesized as described previously(7,10)) was added to 1.5 mm^2 sections of PLLA. A heparin/growth factor solution containing 1 μL 1.0 mg/mL recombinant murine VEGF-165 (PeproTech), 1 μL 1.0 mg/mL recombinant human FGF-2 (PeproTech), and 4 μL 20 mg/mL aqueous heparin (Sigma) was then added to gel the HBPA solution. Lastly, 1 μL aqueous HBPA was added to the top of scaffolds (scaffold microstructure shown in Supplementary Figure S1 online).

Gelled scaffolds were placed on the omentum of each transplant recipient. Islets (250/recipient) were delivered adjacently to scaffolds, and the omentum was folded over the scaffolds and islets. Test group recipients received islets and HBPA scaffolds containing growth factors as described above (**HBPA-GF**; *n*=9). Control recipients received an equivalent number of islets (250) under the following conditions: **(a) HBPA-CNTRL** (*n*=10): HBPA scaffolds gelled with heparin (no GFs), placed adjacent to omental islet transplants; **(b) OM** (*n*=5): omental islet transplants without scaffolds; **(c) OM-GF** (*n*=5): omental islet transplants with local administration of free growth factors (no scaffolds); **(d) IP** (*n*=6): free islets placed in the peritoneal cavity; **(e) IH** (*n*=5): intrahepatic islet implantation via portal venous delivery (no scaffolds, no GFs); **(f) KC** (*n*=6): islets implanted in the subcapsular space of the kidney (no scaffolds, no GFs). In a separate study to assess transplant site vascularization, **HBPA-GF** (*n*=6) and **HBPA-CNTRL** (*n*=6) scaffolds were implanted separately into the omenta of male FVB/N mice and analyzed via histochemical procedures for the presence of CD31, an endothelial-specific marker. Another set of **HBPA-GF** (*n*=3) and **HBPA-CNTRL** (*n*=3) scaffolds were implanted into omenta of FVB/N-Tg(*Vegfr2-luc*) transgenic mice (Xenogen Corp.)(17) which have a modified *VEGFR2* (VEGF receptor 2) promoter to couple *VEGFR2* gene expression to a luciferase reporter. Potential post-implantation angiogenic activity related

to VEGFR2 up-regulation in existing or proliferating cells was monitored noninvasively by injecting subjects with 150 mg/kg intraperitoneal luciferin and viewing them with an IVIS imaging system (Xenogen).

Implantation studies indicated that **HBPA-GF** scaffolds induced significant biological responses in the surrounding tissue (Figure 1; see Supplementary Figure S2 online for images of the response at an alternative site). Mean densities of CD31-positive neovessels were nearly eight times greater in histochemical sections of **HBPA-GF** scaffolds than in sections of **HBPA-CNTRL** scaffolds, which did not contain growth factors (Figure 1d; $P=2.86\times10^{-4}$). The distribution of neovessels throughout the scaffolds and immediately surrounding omental tissue suggested that omental islets in **HBPA-GF** transplants were within close proximity to newly formed vessels. The presence of intralumenal erythrocytes (Figure 1c) in these vessels is consistent with early stage angiogenesis and implies basic vessel functionality(18). These outcomes are consistent with our previous findings pertaining to HBPA-delivered VEGF/FGF-2 in a rat cornea angiogenesis model(10).

Bioluminescent images of HBPA scaffolds implanted in *Vegfr2-luc* transgenic mice provided additional evidence of the biological activity of scaffold-derived growth factors. **HBPA-GF** scaffold recipients generally showed stronger bioluminescent signals than **HBPA-CNTRL** recipients (Figure 2). Signal intensities in these images are spatially and temporally correlated to the up-regulation of VEGFR2, a primary VEGF receptor on endothelial cell surfaces that mediates the growth factor's mitogenic, survival, and permeability effects that are essential to angiogenesis(19). Signals in **HBPA-GF** recipients appeared 2–3 days post-implantation and diminished over the course of 10–14 days, thus indicating the kinetics of the biological response to growth factors released from **HBPA-GF** scaffolds.

The increased vascular density in **HBPA-GF** scaffolds was associated with a significant improvement in islet functional outcomes (Figure 3; see Supplementary Figure S3 online for a sample recipient blood glucose profile). Specifically, the proportion of **HBPA-GF** recipients which achieved normoglycemia (78%) was over 2.5 times greater than that of controls which received identical scaffolds without growth factors (**HBPA-CNTRL**; 30%)($P=0.037$) or free growth factors without scaffolds (**OM-GF**; 20%). **HBPA-GF** recipients also achieved normoglycemia in significantly shorter times than **HBPA-CNTRL** recipients ($P=0.027$). Collectively, these findings confirm the bioactivity of the **HBPA-GF** scaffolds and suggest that their positive impact on transplant outcomes relates—at least in part—to their ability to modify the omental microenvironment in ways that make it more suitable for islet engraftment.

The critical role of the HBPA-delivered VEGF and FGF-2 in facilitating islet function is evident in the low rates of normoglycemia in the **HBPA-CNTRL** and **OM** groups, where growth factors were not used. These outcomes indicate that the unmodified omentum is marginally suited for islet transplantation and that HBPA scaffolds had relatively little effect on transplanted islets in the absence of the growth factors. On the other hand, the low cure percentages of the **OM-GF** group show that bolus growth factor delivery without scaffolds is also ineffective. This indicates that HBPA scaffolds play an important role in growth factor storage and delivery. Thus, the enhanced engraftment in **HBPA-GF** transplants can be attributed to specific growth factor effects that were made possible by the growth factor delivery mechanism of the HBPA nanostructures. This outcome is consistent with previous findings in a rat cornea angiogenesis assay, where it was shown that **HBPA-GF** scaffolds produced significantly stronger angiogenic responses than either direct growth factor administration or delivery by other carriers such as collagen or heparin(10).

The transplant outcomes were observed in three ways: 1) some **HBPA-GF** recipients achieved immediate islet function that was not observed in **HBPA-CNTRL** or **OM-GF** recipients, 2)

some **HBPA-GF** recipients achieved islet function one-to-two weeks earlier than **HBPA-CNTRL** or **OM-GF** recipients, and 3) some **HBPA-GF** recipients achieved islet function at later intervals post-transplant. This was an interesting observation that will require further evaluation to discern whether there are multiple mechanisms to the engraftment process. Considering that post-transplant islet revascularization requires 7–14 days, these results raise the possibility that the HBPA-delivered VEGF and FGF-2 may have had additional beneficial effects beyond transplant site revascularization. Support for this hypothesis can be found in the fact that VEGF, FGF-2, and their receptors are expressed in both developing and mature islets(12–14,20,21), even though angiogenic activity diminishes greatly after initial development. VEGF is present in elevated levels inside healthy islets and has been implicated in post-transplant preservation of β-cell mass(18,22,23) and the maintenance of vascular features required for proper function(12,15). Similarly, FGF signaling has been shown to play important roles in islet morphogenesis(13), insulin processing(14), glucose sensing(14), and β-cell differentiation(13) and proliferation(14). This hypothesis is further supported by recent evidence suggesting important relationships between β-cells and intraislet endothelial cells (24).

In conclusion, this study provides the first demonstration of HBPA nanofiber signaling efficacy in a functional transplant model. HBPA-based delivery of VEGF and FGF-2 to the omental transplant site significantly increased vascular density ($P<0.0005$) *and* improved islet engraftment, as evidenced by significantly higher cure percentages ($P<0.05$) and significantly shorter times to achieve normoglycemia ($P<0.05$). While the **HBPA-GF** cure percentage did not surpass that of the **IH** group, the results illustrate the potential utility of HBPA scaffolds in developing alternatives to intrahepatic implantation. Alternative islet engraftment sites can potentially address concerns associated with portal venous puncture and post-engraftment liver damage(25–27), and may offer additional advantages, such as the accommodation of larger volumes of tissue(4) and insulin delivery via the portal venous circulation(28).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

Abbreviations

PA
: peptide amphiphile

HBPA
: heparin-binding peptide amphiphile

ECM
: extracellular matrix

VEGF
: vascular endothelial growth factor

FGF-2
: fibroblast growth factor 2

PLLA
: poly(L-lactic acid)

CD31

cluster determinant 31 (also called platelet endothelial cell adhesion molecule-1, PECAM-1)

t_{cure}
cure time; duration needed to achieve sustained normoglycemia, measured in days post-transplant

VEGFR2
vascular endothelial growth factor receptor 2 (also KDR/FLK-1)

AUC
area under curve for plot of cumulative percentage cured vs. time

Transplant designations (see Fig. 3a)

HBPA-GF
islets wrapped in the omentum adjacent to HBPA scaffolds containing growth factors (VEGF, FGF-2)

HBPA-CNTRL
islets wrapped in the omentum adjacent to HBPA scaffolds without growth factors

OM
islets wrapped in the omentum without scaffolds

OM-GF
islets wrapped in the omentum with free growth factors (no scaffolds)

IP
free islets introduced into the peritoneal cavity via Pasteur pipette

IH
free islets injected into the hepatic portal vein (single injection)

KC
centrifuged islet pellets introduced into the subcapsular space of the kidney

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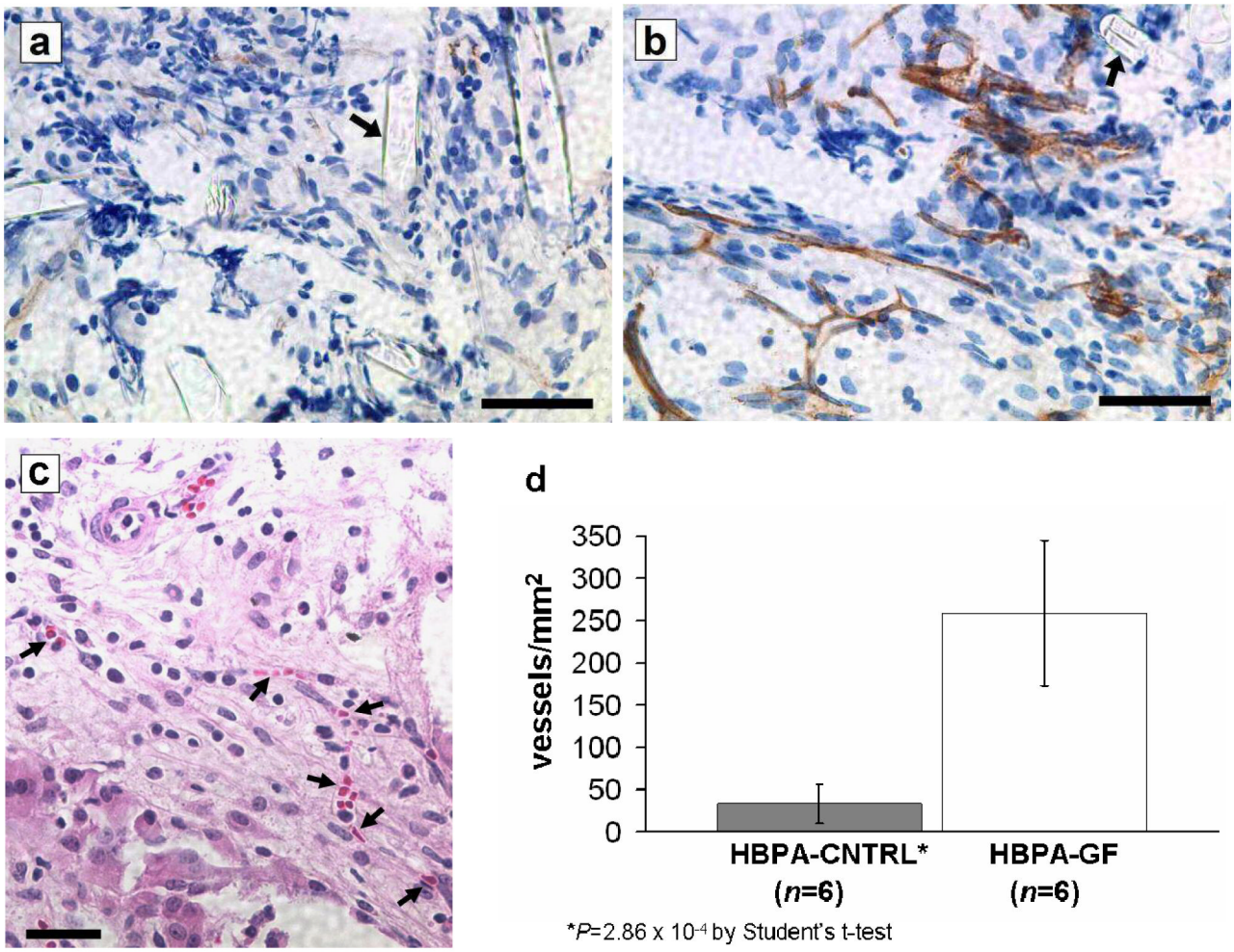

Figure 1. VEGF and FGF-2 delivered via HBPA nanostructures significantly increase vascular density at the omental transplant site

(a,b) CD31 staining of **(a) HBPA-CNTRL** and **(b) HBPA-GF** scaffolds retrieved from omenta on post-transplant day 14. CD31 positive cells are stained brown by DAB chromogen; cell nuclei are stained blue by hematoxylin. Arrows denote sections of PLLA filaments amongst the infiltrating cellular tissue (scale bars represent 25 μm). **(c)** Hematoxylin and eosin staining of an **HBPA-GF** scaffold retrieved on day 14. Erythrocytes in the lumens (see arrows) suggest that neovessels have functional characteristics (scale bar represents 30 μm). **(d)** Density of CD31-positive neovessels in HBPA scaffold specimens retrieved between days 11 and 14. Neovessel densities in **HBPA-GF** specimens were nearly 8 times greater than those in **HBPA-CNTRL** specimens (error bars represent 95% confidence intervals).

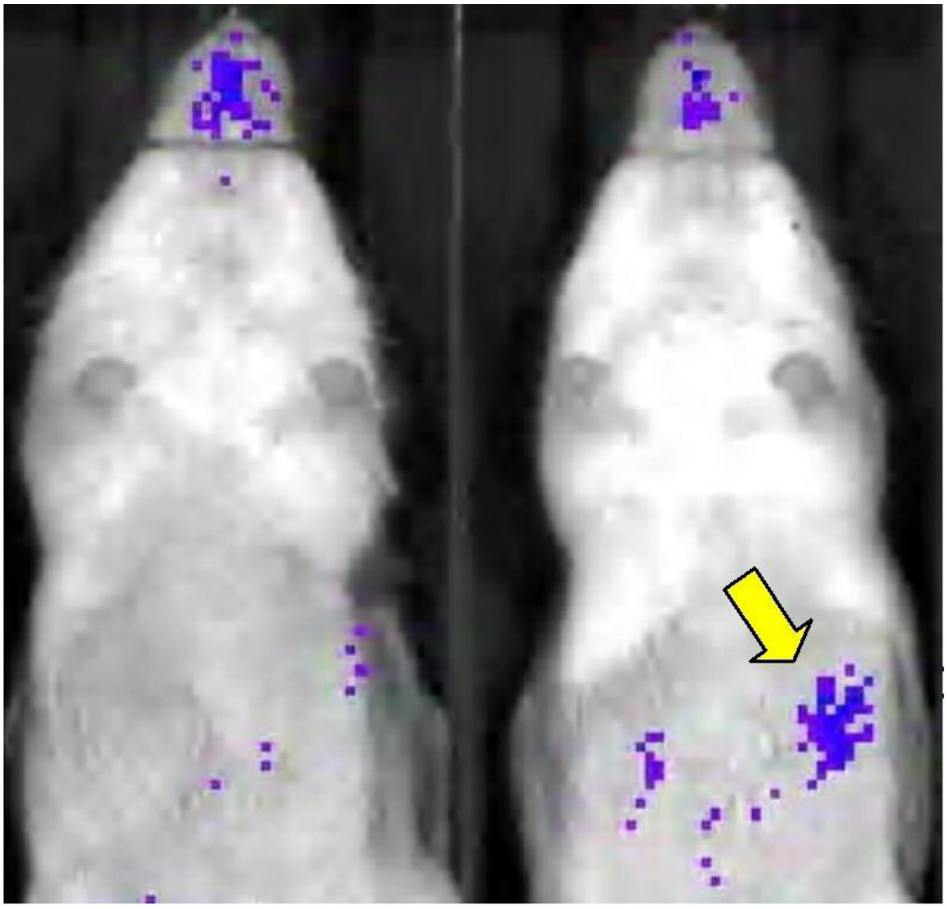

Figure 2. Bioluminescent signals in *vegfr2-luc* transgenic mice provide additional, noninvasive evidence of the biological activity of HBPA-GF scaffolds

The **HBPA-GF** recipient (right) shows considerably greater bioluminescence due to growth factor-induced VEGFR2 expression than the **HBPA-CNTRL** recipient (left). The yellow arrow indicates the location of the omental bioluminescent signal in the **HBPA-GF** recipient.

a

Group	n	site	scaffold	growth factors	t_{cure} (d) (*non cures in parentheses*)	mean t_{cure} ± SD	%cure
IH	5	intrahepatic	--	--	0.5 x 3, 1.5, 3.5	**1.3 ± 1.3**	**100**
KC	6	kidney capsule	--	--	0.5 x 5, 4.5	**1.2 ± 1.6**	**100**
IP	6	intraperitoneal	--	--	*(80 x 6)*	--	**0**
OM	5	omentum	--	--	1.5 *(80 x 4)*	--	**20**
OM-GF	5	omentum	--	VEGF/FGF-2 (free)	27 *(80 x 4)*	--	**20**
HBPA-CNTRL	10	omentum	HBPA	--	37, 51, 68 *(80 x 7)**	**52 ± 15.5**	**30****
HBPA-GF	9	omentum	HBPA	VEGF/FGF-2	0.5 x 2,12,12.5,41,58,65 *(80 x 2)**	**27.1 ± 27.2**	**78****

**P* = 0.027 by two-tailed Mann-Whitney-Wilcoxon Rank Sum test
***P* = 0.037 by Pearson's Chi-square test

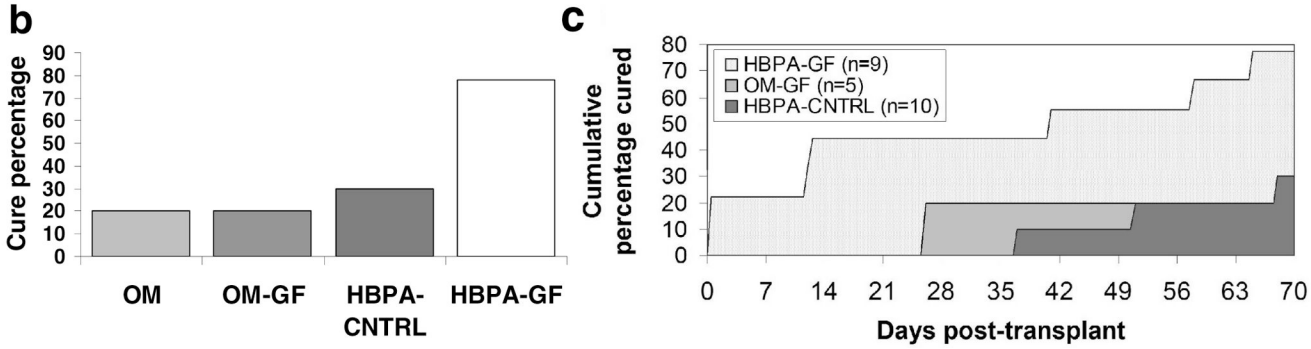

Figure 3. Effects on transplant outcome of VEGF and FGF-2 delivered via HBPA nanostructures

(a) Group outcomes for transplants of 250 islets. $\mathbf{t_{cure}}$ represents the post-transplant time (days) in which a streptozotocin-induced diabetic recipient achieved sustained normoglycemia. **(b)** Comparison of the proportions of islet isograft recipients that ever achieved normoglycemia using the omental transplantation site. **(c)** Comparison of the proportions of islet isograft recipients that achieved normoglycemia according to time post-transplant. The area under each curve (AUC) provides a visual metric of transplant effectiveness. Cure percentages and $\mathbf{t_{cures}}$ for omental transplants improved considerably when islets were delivered with HBPA scaffolds containing growth factors (**HBPA-GF**), as indicated by the large AUC. Islets delivered with either unbound growth factors (**OM-GF**) or plain HBPA scaffolds without growth factors (**HBPA-CNTRL**) had substantially smaller AUCs and showed little difference in performance over the OM and IP controls.