Engineering Bioactive Nanoparticles to Rejuvenate Vascular Progenitor Cells

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1 **Engineering Bioactive Nanoparticles to Rejuvenate** 2 **Vascular Progenitor Cells**

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30 **Abstract**

31 Fetal exposure to gestational diabetes mellitus (GDM) predisposes children to future health
32 complications including type-2 diabetes mellitus, hypertension, and cardiovascular disease. A
33 key mechanism by which these complications occur is through stress-induced dysfunction of
34 endothelial progenitor cells (EPCs), including endothelial colony-forming cells (ECFCs).
35 Although several approaches have been previously explored to restore endothelial dysfunction,
36 their widespread adoption remains hampered by systemic side effects of adjuvant drugs and
37 unintended immune response of gene therapies. Here, we report a strategy to rejuvenate
38 circulating vascular progenitor cells by conjugation of drug-loaded liposomal nanoparticles
39 directly to the surface of GDM-exposed ECFCs (GDM-ECFCs). Bioactive nanoparticles can be
40 robustly conjugated to the surface of ECFCs without altering cell viability and key progenitor
41 phenotypes. Moreover, controlled delivery of therapeutic drugs to GDM-ECFCs is able to
42 normalize transgelin (TAGLN) expression and improve cell migration, which is a critical key
43 step in establishing functional vascular networks. More importantly, sustained pseudo-
44 autocrine stimulation with bioactive nanoparticles is able to improve *in vitro* and *in vivo*
45 vasculogenesis of GDM-ECFCs. Collectively, these findings highlight a simple, yet promising
46 strategy to rejuvenate GDM-ECFCs and improve their therapeutic potential. Promising results
47 from this study warrant future investigations on the prospect of the proposed strategy to
48 improve dysfunctional vascular progenitor cells in the context of other chronic diseases, which
49 has broad implications for addressing various cardiovascular complications, as well as
50 advancing tissue repair and regenerative medicine.

51 **Introduction**

52 Cardiovascular disease is the most prevalent cause of mortality and morbidity among
53 patients with diabetes.¹ Adults with diabetes have a two to six times higher risk of developing
54 cardiovascular disease than unaffected individuals.² Similarly, fetal exposure to gestational
55 diabetes mellitus (GDM) predisposes children to future health complications including type 2
56 diabetes mellitus (T2DM), hypertension, and cardiovascular disease.^{3,4} Although the
57 pathophysiology that links diabetes and cardiovascular disease is complex and multifactorial,
58 there is a general agreement that hyperglycemia and oxidative stress lead to stress-induced
59 early endothelial dysfunction,⁵ which is responsible for both macrovascular (i.e., peripheral
60 artery disease, stroke) and microvascular (i.e., diabetic nephropathy, retinopathy)
61 complications.^{6–8} Several pre-clinical and clinical trials are exploring the therapeutic effect of
62 stem and progenitor cell therapies to repair the damaged endothelium and promote
63 neovascularization.^{9–11} One promising autologous cell source is endothelial colony-forming
64 cells (ECFCs), a subtype of endothelial progenitor cells (EPCs), identified from circulating adult
65 blood and highly enriched in human umbilical cord blood.^{12,13} As putative EPCs, these ECFCs
66 express robust proliferative potential in forming secondary and tertiary colonies, as well as *de*
67 *novo* blood vessel formation *in vivo*.¹² Nonetheless, hyperglycemia and a diabetic intrauterine
68 environment also cause premature senescence and significant dysfunction of ECFCs, which
69 limit their therapeutic use.^{3,14,15} ECFCs isolated from patients with diabetes demonstrate
70 delayed colony formation, reduced cell migration, and impaired angiogenic potential.^{3,14,16}
71 Therefore, restoring dysfunctional ECFCs could improve their vasculogenic potential and serve
72 as biomarkers to assess cardiovascular disease risk.^{9,10}

73 Several approaches have been explored to rejuvenate ECFCs and to restore their
74 therapeutic potential. These approaches include the delivery of adjuvant drugs to improve

75 immobilization of progenitor cells, genetic modification of cells to overexpress growth factors,
76 and pre-conditioning of these cells with pharmacological agents.^{17–20} Nonetheless, adjuvant
77 agents need to be maintained at high and sustained systemic levels for efficacy, while
78 genetically modified cells pose high regulatory and cost barriers, which altogether hinder their
79 clinical implementation.²¹ Efforts to enhance the stability and effective presentation of bioactive
80 molecules for improving the therapeutic potential of ECFCs have included attempts to
81 judiciously conjugate vascular endothelial growth factor (VEGF) onto the surface of
82 microparticles,²² as well as presentation of proteins and glycomimetic agents to the cell
83 surface.^{16,17,23,24} Despite recent progress in these areas,²⁵ translational challenges persist for
84 rejuvenating EPCs using growth factor and gene therapies, including unintended immune
85 response, enzymatic degradation, and uncertain toxicology.^{26–28}

86 We report a class of liposomal nanoparticles with tunable release kinetics, which can be
87 conjugated directly onto the cell surface to improve the therapeutic potential of ECFCs isolated
88 from infants born to women with gestational diabetes mellitus (GDM-ECFCs). This work builds
89 on the discovery that GDM-ECFCs have decreased vasculogenic potential and altered gene
90 expression, particularly transgelin (*TAGLN*),^{3,29} also known as smooth muscle protein 22 α
91 (SM22 α). We previously reported that increased *TAGLN* expression in GDM-ECFCs is
92 associated with disrupted actin cytoskeletal rearrangement, which results in reduced cell
93 migration and impaired vasculogenesis.^{3,29,30} Since *TAGLN* is a direct target of the TGF-
94 β /Smad3 pathway,³¹ we hypothesized that delivering TGF- β inhibitor (SB-431542) directly to
95 the surface of the cells could normalize *TAGLN* expression and eventually improve cell
96 migration, which is a critical key step in establishing functional vascular networks. Liposomal
97 formulation of nanoparticles was selected for the fabrication technique because it can form
98 multilamellar structures with high encapsulation efficiency and exhibits controllable release

99 kinetics.^{32,33} Moreover, since a similar formulation of liposomal nanoparticles has been
100 previously used to deliver adjuvant drugs to hematopoietic stem and progenitor cells (HSPCs)
101 without adverse immune response,^{21,34} the utilization of liposomal nanoparticles can ease the
102 clinical translation of our strategy.³⁵

103 We demonstrate that such lipid-based nanoparticles can robustly bind onto the surface
104 of GDM-ECFCs without altering cell viability and key ECFC phenotypes. More importantly,
105 bioactive nanoparticles can significantly normalize *TAGLN* expression, restore cell migration,
106 as well as improve vasculogenesis *in vitro* and *in vivo*. Collectively, with localized and sustained
107 pseudo-autocrine stimulation of therapeutic cells with bioactive nanoparticles, we present a
108 potentially promising strategy to rejuvenate GDM-ECFCs and improve their therapeutic
109 potential to regenerate the vasculature and address a range of cardiovascular complications
110 that precipitate from GDM.

111 **Results**

112 **Engineering bioactive nanoparticles to the cell surfaces**

113 Based on our previous findings that *TAGLN* inhibition can enhance the functionality of
114 GDM-ECFCs,^{14,29} we designed a nanocarrier system to deliver small molecule SB-431542
115 (TGF- β inhibitor) for sustained pseudo-autocrine stimulation to therapeutic cells (**Fig. 1A**).
116 Utilizing a lipid formulation that includes thiol-reactive maleimide headgroups,³⁴ multilamellar
117 lipid nanoparticles with a desired size of 150 nm in diameter were engineered to control the
118 release of bioactive agents (**Fig. 1B** and **Supplementary Fig. 1**). The targeted size and
119 multilamellar structures of the nanoparticles were chosen because previous studies with these
120 similar parameters showed high encapsulation efficiencies and minimal inflammatory
121 responses following *in vivo* implantation.³⁴ Using Dynamic Light Scattering (DLS) and

122 Nanosight measurements, we confirmed that the multilamellar lipid nanoparticles with $147 \pm$
123 63 nm diameter were stable for at least 30 days at both 4°C and 37°C (**Fig. 1C-D** and
124 **Supplementary Fig. 2**), which is suitable for long-term storage and clinical applications. To
125 stably couple bioactive nanoparticles to the surface of ECFCs, we exploited the high level of
126 reduced thiol groups present on the surface of many progenitor cells.^{36,37} First, we detected
127 substantial amounts of free thiols on ECFCs (**Supplementary Table 1**). Despite some
128 variations between biological replicates from different patient samples, there were no
129 significant differences between the level of free thiols among normal ECFCs and GDM-ECFCs
130 ($p>0.688$; **Supplementary Fig. 3**). Next, nanoparticles were conjugated to the surfaces of
131 ECFCs using a benign maleimide-thiol coupling, followed by *in situ* PEGylation to quench
132 residual reactive groups of the nanoparticles (**Fig. 1A**).³⁷ Upon conjugation, the nanoparticles
133 were successfully attached to the cell surfaces (**Fig. 1E**). Increasing the ratio of cells to
134 nanoparticles from 1:100 to 1:5,000 correlates well with the increase in the mean fluorescence
135 intensity (MFI) as detected by flow cytometry analysis (**Fig. 1F**). We determined that 5,000
136 (± 100) was the maximum number of particles (without encapsulated drug cargo) that can be
137 conjugated on the surface of ECFCs without effecting cell viability and proliferation (**Fig. 1G**).
138 These findings are consistent with previous studies reporting that attachment of nanoparticles,
139 each 150 nm in diameter, would occlude only 5% of the surface of a typical 20- μ m-diameter
140 cell.^{34,37} Moreover, nanoparticles could be loaded with various concentrations of the
141 hydrophobic small molecule (SB-431542) and demonstrated consistent week-long drug
142 release profiles (**Fig. 1H**). By fitting the drug release kinetic to the Korsmeyer-Peppas model,³⁸
143 n values of 0.52 to 0.63 were obtained, suggesting that non-Fickian diffusion is the main driving
144 force of drug release (**Fig. 1H** and **Supplementary Fig. 4**). Based on these findings and
145 previous studies that reported a lack of an inflammatory response from innate immune cells

146 exposed to the nanoparticles,^{34,37} we utilized these lipid multilamellar nanoparticles (up to
147 1:5,000 ratio) loaded with 40 μ m SB-431542 for our subsequent *in vitro* functionality and *in vivo*
148 therapeutic studies.

149 **Conjugation of nanoparticles does not alter ECFC phenotypes**

150 Once the optimum nanoparticle properties and parameters were determined, we next
151 tested if nanoparticle conjugation altered key ECFC functions and phenotypes. ECFCs
152 conjugated with nanoparticles up to 5,000 ($\pm$ 100) per cell retained a robust proliferative
153 potential that was comparable to unconjugated control cells (**Fig. 2A-D**). During cell division,
154 nanoparticles attached to the surface of ECFCs segregated equally to their daughter cells, as
155 reflected by a stepwise decrease in the MFI from ECFC-conjugated nanoparticles,
156 corresponding with sequential divisions over several days (**Fig. 2C-D** and **Supplementary Fig.**
157 **5**). Furthermore, we also assessed the impact of nanoparticles conjugation on cell surface
158 markers specific to ECFCs.^{9,12} Comparative flow cytometry analysis revealed that expression
159 levels of CD31, CD34, and CD144 were comparable between normal ECFCs and GDM-ECFCs
160 (**Fig. 2E-G** and **Supplementary Fig. 6**). Despite a slight variation in CD31 expression among
161 the biological replicates, there was no statistical difference between them ($^{\#}P = 0.387$). In
162 addition, nanoparticle conjugation did not alter expression of CD31, CD34, and CD144 for
163 normal ECFCs and GDM-ECFCs (**Fig. 2H-J** and **Supplementary Fig. 6**). Collectively, these
164 data suggest that conjugation of nanoparticles does not significantly alter key functions and
165 phenotypes of ECFCs.

166 **Drug loaded nanoparticles normalize TAGLN expression**

167 Because *TAGLN* is a TGF- β inducible gene that contributes to GDM-ECFC
168 dysfunction,¹⁹ we investigated whether nanoparticles loaded with SB-431542 and conjugated

169 to the surface of ECFCs can stably normalize *TAGLN* expression. First, we confirmed using
170 qRT-PCR that GDM-ECFCs cultured in media supplemented with 5 μ M of SB-431542 for 72
171 hr demonstrated a decrease in the relative mRNA expression of *TAGLN* (**Fig. 3A**). However,
172 the SB-431542-mediated reduction of *TAGLN* expression in GDM-ECFCs was transient and
173 unstable (**Fig. 3B**). Upon removal of the SB-431542 from the culture media of GDM-ECFCs
174 (after 72 hr), relative *TAGLN* mRNA expression increased to levels comparable to *TAGLN*
175 expression in vehicle control GDM-ECFCs at day 5 and 6 (**Fig. 3B**). These findings are
176 consistent with previous studies that showed continuous SB-431542 supplementation in the
177 media is required for the expansion and maintenance of ECs derived from human pluripotent
178 stem cells (hPSCs).^{39–41} Motivated by these observations, we tested if SB-431542-loaded
179 nanoparticles (SB-NPs) conjugated to the surface of ECFCs can provide a pseudo-autocrine
180 stimulation to stably normalize *TAGLN* expression. Indeed, *TAGLN* mRNA levels were reduced
181 in GDM-ECFCs conjugated with the SB-NPs compared to vehicle controls (Vh-NPs). The
182 decrease in *TAGLN* expression appeared to be stable for at least after 6 days in culture (**Fig.**
183 **3C**). Significantly, the reduction in *TAGLN* gene expression correlated well with the decrease
184 in protein expression of *TAGLN* (**Fig. 3D**). Although there were variations in *TAGLN* expression
185 among biological replicates, which correlated well with GDM severity in the patient population
186 (**Supplementary Table 1**), western blot analysis revealed that protein expression of *TAGLN*
187 was significantly lower for GDM-ECFCs conjugated with SB-NPs compared to Vh-NPs controls
188 (**Fig. 3E** and **Supplementary Table 2**). These results underscore the stability of *TAGLN*
189 inhibition using SB-NPs as a critical enabling step in improving the therapeutic potential of
190 GDM-ECFCs.

191 **Bioactive nanoparticles improve cell migration *in vitro***

192 Since TAGLN is an actin binding protein implicated in regulating cell migration, a critical
193 step in establishing vascular networks,^{14,29} we next investigated if SB-NPs could improve cell
194 migration and angiogenic potential of GDM-ECFCs. Initial investigations focused on the ability
195 of SB-NPs to improve cell migration in trans-well migration and wound healing assays.
196 Normalizing TAGLN expression in GDM-ECFCs using SB-NPs resulted in a greater number of
197 cells migrating towards the pro-migratory stimulus (**Fig. 4A** and **Supplementary Fig. 7**). Using
198 four biological replicates for each condition, we observed that SB-NPs significantly improve cell
199 migration of GDM-ECFCs ($*P=0.039$), but not normal ECFCs ($\#P=0.955$, **Fig. 4B**). To obtain a
200 kinetic readout of ECFC migration, a wound healing assay was performed using a time-lapse
201 image analysis (**Fig. 4C** and **Supplementary Movie 1-4**). Starting at 8 hr after wound initiation,
202 GDM-ECFCs conjugated with the SB-NPs demonstrated a significant improvement in wound
203 closure, improving until at least 14 h after wound initiation, compared to GDM-ECFCs
204 conjugated with the Vh-NPs control (**Fig. 4D**). Quantification of the wound closure at 14 hr
205 demonstrated that SB-NPs significantly increased wound closure by GDM-ECFCs ($*P=0.041$),
206 but not by normal ECFCs ($\#P=0.988$). Overall, these results suggest that SB-NPs significantly
207 improve cell migration, which was impaired in GDM-ECFCs.

208 **Bioactive nanoparticles augment 2D and 3D vasculogenesis *in vitro***

209 Our next investigations focused on evaluating if the improvement in cell migration could
210 restore the vasculogenic potential of GDM-ECFCs *in vitro*. A vascular tube formation assay in
211 2D on Matrigel was performed for normal and GDM-ECFCs with either Vh-NPs or SB-NPs (**Fig.**
212 **5A** and **Supplementary Movie 5-8**). We utilized the kinetic analysis of vasculogenesis (KAV),
213 which was extensively characterized in our previous studies,^{29,30,42,43} to quantitate large time-
214 lapsed image data sets and provide high-throughput vasculogenic analysis. While other key
215 parameters (e.g., tube length, vessel area) were quantified, we primarily focused on closed

networks because our previous studies have identified this parameter as the most significant phenotypes effected by intrauterine GDM exposure.^{3,30} Similar to previously observed biphasic trends in ECFC network formation kinetics,^{30,42} we confirmed an increasing number of closed networks occurred in *phase 1* (0-5 hr) until network formation peaks at 5 hr, and then a slight decrease in closed networks occurred in *phase 2* (5-10 hr; **Fig. 5B**). Quantification of the closed networks at 5 hr demonstrated that SB-NPs significantly increased vascular closed networks formed by GDM-ECFCs ($**P=0.010$), but not by normal ECFCs ($\#P=0.218$) in comparison to the Vh-NPs corresponding control groups (**Fig. 5C**). Significantly, the numbers of closed networks in GDM-ECFCs conjugated with SB-NPs remained higher throughout the time course compared to GDM-ECFCs conjugated with Vh-NPs (**Fig. 5B**). Moreover, the difference between GDM-ECFCs conjugated with SB-NPs and Vh-NPs were statistically validated using mean kinetic values for the number of closed networks similar to our previous studies.^{29,30,42} This statistical difference is within the pointwise 95% confidence intervals (error bars) and greater than zero throughout the duration of the experiment suggesting functional improvements of SB-NPs on network structure (**Supplementary Fig. 8**).^{29,30,42} Motivated by this promising result, we further evaluated if bioactive nanoparticles could augment vascular tube formation in a 3D collagen/fibronectin gel, which is usually performed as validation before progressing to *in vivo* vasculogenesis studies.^{3,9,44} First, we encapsulate ECFCs or GDM-ECFCs conjugated with either Vh-NPs or SB-NPs into 3D collagen/fibronectin gels.^{3,9,44} After 24 hrs of culture, 3D capillary tube formation was observed within the gels (**Fig. 5D-E** and **Supplementary Fig. 9**). Then, we utilized the KAV method to quantify the vascular closed networks formed within the 3D collagen/ fibronectin gels.³⁰ KAV analysis revealed that SB-NPs significantly increased 3D vascular closed networks formed by GDM-ECFCs ($***P=0.0031$), but not by normal ECFCs ($\#P=0.051$) in comparison to Vh-NPs corresponding control groups

240 (Fig. 5F).^{30,42} Overall, these results suggest that SB-NPs can significantly restore the 2D and
241 3D vasculogenic potential of GDM-ECFCs.

242 **Bioactive nanoparticles restore *in vivo* vasculogenic potential of GDM-ECFCs**

243 A unique characteristic of ECFCs is their ability to form *de novo* blood vessels *in vivo*
244 upon transplantation.^{12,45,46} In preliminary studies, we demonstrated that GDM-ECFCs exhibit
245 reduced functional capacity to form chimeric vessels *in vivo* compared to normal ECFCs
246 (Supplementary Fig. 10A-C). Therefore, we next investigated whether SB-NPs could restore
247 *in vivo* vasculogenic potential of GDM-ECFCs. Early passages (P2-5) of normal ECFCs and
248 GDM-ECFCs conjugated with either Vh-NPs or SB-NPs were encapsulated in
249 collagen/fibronectin gels to allow 3D capillary tube formation (Supplementary Fig. 9).^{3,9,44} After
250 24 hrs of culture, the pre-vascularized grafts were transplanted into each flank of
251 immunodeficient NOD/SCID mice.^{4,44,47} Since SB-NPs do not augment *in vitro* vascular
252 formation of normal ECFCs, *in vivo* studies focused on assessing whether SB-NPs conjugated
253 to GDM-ECFCs improved vasculogenic function *in vivo*. At 14 days, the grafts were harvested
254 and processed for histological and immunohistochemical analysis (Supplementary Fig. 11A-
255 D). Qualitatively, the overall size of the grafts from the GDM-ECFCs conjugated with Vh-NPs
256 was smaller compared to the grafts from the normal ECFCs conjugated with Vh-NPs or GDM-
257 ECFCs conjugated with SB-NPs (Fig. 6A-C). Normal ECFCs vascularized the entire graft,
258 while GDM-ECFCs sparsely vascularized the periphery of the graft (Fig. 6A-B and
259 Supplementary Fig. 10A-C). In contrast, GDM-ECFCs conjugated with SB-NPs vascularized
260 the entire graft, comparable to normal ECFCs conjugated with Vh-NPs (Fig. 6A and C). The
261 majority of chimeric vessels stained positive for human CD31 and perfused with mouse
262 erythrocytes, suggesting graft vessels that were functionally anastomosed with host vessels
263 (Fig. 6D-F and Supplementary Fig. 11A-D). Quantification of the chimeric vessels revealed

264 that SB-NPs conjugation of GDM-ECFCs more than doubled vessel density compared to Vh-
265 NPs conjugation (44.7 ± 11.7 vs. 102.5 ± 46.3 vessels/mm², **Fig. 6G**). Similarly, vessel area
266 significantly increased for GDM-ECFCs conjugated with SB-NPs compared to Vh-NPs
267 conjugation (**Fig. 6H**).

268 To further study the dynamic interaction between the human and host vasculatures,
269 perfusion imaging of the grafts was performed using intravital microscopy. Prior to harvesting
270 the grafts (at day 14), mice were perfused with rhodamine-conjugated *UEA-I* lectin and
271 fluorescein-conjugated *GS-IB4* isolectin to label the human and mouse vessels,
272 respectively.^{44,48,49} Intravital microscopy revealed that host vasculature invaded the periphery
273 of the human vascularized grafts (**Supplementary Fig. 12**). Furthermore, 3D rendering of the
274 grafts demonstrated the dynamic interaction between the human vessels stained with *UEA-I*
275 lectin (human) and the host vasculature stained with *GS-IB4* isolectin (mouse). Chimeric
276 vessels were detected with some overlapping stains between human- and mouse-specific
277 lectins, which suggests that the transplanted human vascular networks were functional and
278 anastomosing with the hosts' circulatory system (**Fig. 6I**). Quantification of the vessels in the
279 grafts revealed that SB-NPs conjugation significantly increased human and mouse vessels that
280 were interconnected to each other (**Fig. 6I-J**). Interestingly, SB-NPs conjugation of GDM-
281 ECFCs also shifted the mean vessel size distribution (10.7 ± 3.6 vs. 14.6 ± 5.8 μ m, **Fig. 6K**).
282 Collectively, these results suggest that SB-NPs significantly improve the *in vivo* vasculogenic
283 potential of GDM-ECFCs.

284 Discussion

285 Our study focuses primarily on fetal ECFCs isolated from infants born to mothers with
286 GDM, which have an increased risk of developing chronic health complications, including

287 T2DM, hypertension, and cardiovascular diseases (**Fig. 7**).^{14,29} Exposure to a diabetic
288 intrauterine environment induces premature dysfunction of ECFCs, which are present in the
289 circulation and in vessel walls, and are highly enriched in umbilical cord blood.^{3,4} A genome-
290 wide microarray analysis conducted on cord blood-derived ECFCs identified *TAGLN* as one of
291 the genes significantly increased in GDM-ECFCs.²⁹ TAGLN is an F-actin binding protein that
292 regulates the organization of actin cytoskeletal, cellular contractility, and motility.^{29,31,50} When
293 TAGLN was first discovered, it was named as SM22 α because it was considered a calponin-
294 related protein expressed specifically in adult smooth muscle cells.^{51,52} Subsequent studies
295 demonstrated TAGLN expression in fibroblasts, epithelial cells, and multipotent mesenchymal
296 stromal cells (MSCs), where TAGLN has a role in generating committed progenitor cells from
297 undifferentiated MSCs by regulating cytoskeletal organization.⁵³

298 Like most healthy ECs, ECFCs express low levels of TAGLN.^{4,8,29} However, exposure
299 to high glucose and inflammatory cytokines induces TAGLN expression through TGF- β and
300 IL1- β signaling pathways.^{29,50} Increased TAGLN expression does not represent trans
301 differentiation of GDM-ECFCs to other cell types, rather it suggests a transition of GDM-ECFCs
302 into an unstable state, which can be reversed with the administration of exogenous
303 therapeutics. In fact, knocking down TAGLN expression with siRNA rescues cell migration and
304 vasculogenic potential of GDM-ECFCs.²⁹ Collectively, these observations provided rationale
305 for evaluating whether a TGF- β inhibitor could enhance the vasculogenic function of GDM-
306 ECFCs.

307 Inspired by pioneering studies that conjugated drug-cargos to the surface of
308 HSPCs,^{37,54,55} we engineered bioactive NPs that can directly attach to the cell surface and
309 deliver therapeutic agents to the GDM-ECFCs. The multilamellar structure of the NPs allows

310 high encapsulation efficiency of the therapeutic reagents (above 70%), long-term stability, and
311 sustained release via diffusion up to 14 days, which matches the timeline required for ECFCs
312 to undergo vascular anastomosis *in vivo*.^{44,47,48} Similar to other circulating progenitor cells,^{36,37}
313 we demonstrated that ECFCs express high level of free thiols on the cell surface, which can
314 be used for benign thiol-maleimide coupling. Following NPs conjugation to the cell surface, the
315 residual reactive groups on the NPs are quenched using a PEGylation strategy,^{56,57} which has
316 been widely used for improving NPs-based drug delivery.^{58,59} Given that the size of a typical
317 ECFC is larger than HSPC, we demonstrated that we can conjugate up to 5,000 NPs per cell,
318 while preserving cellular function and progenitor phenotypes of ECFCs. Moreover, the
319 engineered NPs provide a sustained and controlled release of SB-431542, which acts as an
320 inhibitor of the TGF- β /SMAD3 pathway. By competing for the ATP binding site, SB-431542
321 selectively inhibits the TGF- β type I receptor ALK5, whereas it does not affect the BMP type 1
322 receptors like ALK2, ALK3, ALK6.⁶⁰ Selective targeting of the ALK5/SMAD3 pathway can
323 directly downregulate TAGLN expression.³¹

324 We have previously utilized siRNA to downregulate TAGLN expression in GDM-ECFCs,
325 which resulted in the improvement of cell migration and vascular tube formation *in vitro*.²⁹ In
326 this study, we tested if SB-NPs conjugated to the surface of GDM-ECFCs can improve the
327 clinical potential of GDM-ECFCs, without the unanticipated vascular and immune effects
328 associated with siRNA therapeutics.²⁸ To evaluate our regenerative strategy, we conducted a
329 3D vasculogenesis assay, where ECs have been shown to form functional vascular networks
330 with lumen structures within collagen/fibronectin gels.^{9,61,62} Using KAV analysis to quantify the
331 vascular networks in 3D collagen/fibronectin constructs,^{30,42} we demonstrated a significant
332 increase in the closed networks formed by GDM-ECFCs conjugated with SB-NPs compared to
333 GDM-ECFCs conjugated with Vh-NPs control. It is interesting to note that SB-NPs conjugated

334 to normal ECFCs does not significantly affect cell migration or vascular tube formation, which
335 may be because these cells express normal TAGLN levels.^{14,29} It is possible that the improved
336 functional effects observed in GDM-ECFCs with SB-NPs were not entirely due to changes in
337 TAGLN expression, since TGF- β inhibition may effect expression of other proteins in ECFCs.⁶³
338 Nonetheless, these data are encouraging and support the notion that specifically targeting
339 disrupted molecular pathways may reduce off-target effects of normal cells *in vivo*.⁶⁴ Moreover,
340 given that improvements in the functional properties of GDM-ECFCs is associated with reduced
341 TAGLN expression, TAGLN could serve as a molecular marker to screen for future small
342 molecule compounds.

343 Since the hallmark of ECFCs is their ability to form *de novo* blood vessel formation *in*
344 *vivo*, our next investigations focused on determining if SB-NPs can restore *in vivo* vasculogenic
345 potential of GDM-ECFCs.^{12,46,65} The subcutaneous xenograft model employed in this study
346 creates a consistent and robust vasculogenic response by ECFCs.^{12,45,49,66–68} As such, this
347 model is the preferred small animal study to compare vasculogenic potential among ECFCs
348 from different cell sources or experimental treatments.^{3,14,69,70} Hence, based on previous
349 studies that analyzed the kinetics of human and host vessels integration,^{44,47,48,71} the xenografts
350 were analyzed using intravital microscopy and immunohistochemistry after 14 days of
351 implantation. We observed that GDM-ECFCs were only able to sparsely vascularize the
352 periphery of the grafts, which may explain why the grafts containing GDM-ECFCs were
353 relatively smaller in size compared to grafts containing normal ECFCs. Interestingly, SB-NPs
354 conjugation significantly increased the vessel density and vessel areas formed by GDM-ECFCs
355 *in vivo*, as well as restored their vasculogenesis potential to a comparable level to normal
356 ECFCs.^{9,72} Notably, the majority of the chimeric vessels formed within the grafts were stained
357 positive for human CD31 and perfused with mouse erythrocytes, which suggests functional

358 vessels that were anastomosed with the host vessels.^{9,68,72} Further quantification of the percent
359 area covered by vessels in the grafts revealed that SB-NPs conjugation resulted in a significant
360 increase of both human vessels and mouse vessels that were interconnected to each other.
361 Since it was previously shown that the host vasculature can connect with the human
362 vasculature through the “wrapping and tapping” mechanism,⁴⁸ the increased in both human
363 and host vessels in the grafts indicate a functional chimeric vascular networks that were able
364 to integrate with the host vasculatures. Altogether, these results suggest that SB-NPs can
365 significantly improve the *in vivo* vasculogenic potential of GDM-ECFCs.

366 We report a simple, yet promising strategy to conjugate bioactive NPs directly onto the
367 surface of ECFCs to improve their vasculogenic potential (**Fig. 7**).^{22,73} Promising results from
368 this study warrant future investigations on the prospect of the proposed strategy to improve
369 ECFC function in other clinically-relevant models, such as peripheral artery disease and
370 diabetic non-healing wounds.^{73–75} Moreover, such a simple cell-surface engineering strategy
371 can be broadly applied to improve dysfunctional ECFCs from the peripheral blood of patients
372 with chronic diseases (i.e., T2DM)^{3,16} and from umbilical cord blood of patients with complicated
373 pregnancies (i.e., preeclampsia),^{69,70,76} as well as to improve the expansion and maintenance
374 of vascular progenitor cells derived from hPSCs.^{17,39–41} This approach also has the potential
375 to increase autologous donor cell pools by enhancing the functional capacity of progenitor cells
376 previously considered unsuitable for cell therapy.^{77,78} Collectively, our study demonstrates a
377 promising platform to rejuvenate vascular progenitor cells, which can be clinically-translated to
378 improve non-invasive, cellular based treatments for cardiovascular complications and to
379 enhance current approaches for tissue repair and regenerative medicine.^{11,25}

380

381 **Materials and Methods**

382 **Umbilical Cord Blood Sample Acquisition**

383 Human umbilical cord blood samples (40-60 mL) were collected in heparinized solution
384 at the time of birth for normal / uncomplicated and GDM pregnancies (gestational age 38-42
385 weeks) following written informed consent.^{4,14} GDM was defined per American College of
386 Obstetrics and Gynecology guidelines. Exclusion criteria include T1DM or T2DM, illness known
387 to affect glucose metabolism (i.e., Cushing syndrome, polycystic ovarian syndrome), use of
388 medications that affect glucose metabolism (i.e., dexamethasone), multiple gestation, history
389 of pre-eclampsia, cardiovascular disease, and women carrying fetuses with chromosomal
390 abnormalities.^{14,79} Historical and clinical data were obtained at each visit and at the time of
391 delivery (**Supplementary Table 1**). Maternal blood was collected for glycosylated hemoglobin
392 (HgA1C) and oral glucose tolerance test (GTT). Cord blood was collected and processed in
393 our AngioBioCore facility for human mononuclear cells (MNCs) used for ECFC isolation and
394 assays. The Institutional Review Board at the Indiana University School of Medicine (IUSM)
395 approved all protocols, and informed consent was obtained from all women.

396 **Isolation and characterization of ECFCs**

397 The human ECFCs were isolated and characterized as previously described.^{3,9} Briefly,
398 tissue culture plates pre-coated with collagen I were seeded with human MNCs in complete
399 endothelial growth medium-2 (EGM-2). After 24 hours of culture, nonadherent cells were
400 aspirated and complete EGM-2 medium was added to each well. Colonies of endothelial cells
401 appeared between 5 and 8 days and were identified as monolayers of cobblestone-appearing
402 cells. ECFCs were characterized for the positive expression of cell-surface antigens CD31,
403 CD141, CD105, Cd144, vWF, and Flk-1, as well as negative expression of hematopoietic-cell
404 surface antigens CD41 and CD14. Single cell colony forming assays were used to characterize

405 their robust and proliferative potential, secondary and tertiary colony formation upon plating.
406 Normal or uncomplicated ECFC lines include E1-CB-111, E1-CB-150, E1-CB-153, E1-CB-157;
407 GDM-ECFC lines include E1-CB-36, E1-CB-37, E1-CB-71, and E1-CB-74 (**Supplementary**
408 **Table. 1**). To maintain the ECFC culture, flasks or well plates were pre-coated with rat-tail
409 collagen type I solution (50µg/mL, Corning), then incubated for at least 3 h at 37°C and washed
410 with PBS three times before use. Complete medium for growing ECFCs consisted of EGM-2
411 (Promocell, C-22011), supplement Mix (Promocell, C-39216), and 0.2% mycoZap™
412 Prophylactic (Lonza). The cells were maintained at 37°C, 5% CO₂, passaged using DetachKit
413 (Promocell, C-41222), and used for experiments between passages 2-5. All cell lines were
414 routinely tested for mycoplasma contamination and were negative throughout this study.

415 **Nanoparticle Fabrication**

416 Synthesis of multilamellar liposomal nanoparticles (NPs) was performed based on the
417 standard thin film lipid hydration method.^{34,37} The components consist of phospholipids
418 (Avanti® Polar Lipids) including MPB-PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-
419 [4-(p-maleimidophenyl)butyramide] (sodium salt)), DOPC (1,2-dioleoyl-sn-glycero-3-
420 phosphocholine), DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt),
421 fluorescent tracker 1,1'-Dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine perchlorate (Dil,
422 Sigma-Aldrich), and SB-431542 (SB, Stemcell Technology) (**Supplementary Fig. 1A**). DOPC,
423 DOPG, MBP-PE, SB-43154 and 'Dil' dye lipid components from Avanti Lipids Inc. were
424 combined in a glass vial and vacuum dried (-25 mm of Hg, 21°C) for 30 to 45 minutes to obtain
425 the dry lipid film. To fabricate liposomal nanoparticles, the dry lipid film containing
426 DOPC/DOPG/MPB-PE/Dil/SB (1188/303/1890/120/3.6µg) was hydrated with 1ml PBS
427 solution. The resulting mixture was vortexed for 5-7 min, then extruded 21 times using gas tight
428 syringes, through a 200nm polycarbonate membrane sandwiched in the Mini-Extruder block

429 (Avanti® Polar Lipids) to improve monodispersity of small liposomal vesicles (<200 nm). The
430 resulting solution was incubated at room temperature for 1.5 hr, then at 4°C overnight. The
431 NPs were purified from free phospholipids and free SB by ultracentrifugation at 50,100 rpm at
432 4°C for 2.5hr (ThermoScientific, Sorvall MX120+ Micro-Ultracentrifuge, Rotor S55-A2). The SB-
433 NPs were obtained by resuspending the pellet in PBS.

434 **Conjugation of Drug-Loaded Nanoparticles to ECFCs**

435 Drug-loaded nanoparticles were conjugated on the surface of the cells by mixing equal
436 volumes of ECFCs and SB-NPs in nuclease-free water, with nanoparticles to cell ratios ranging
437 from 100 to 5,000.^{21,34} The cells were then incubated for 30 min at 37°C with gentle agitation
438 to facilitate the conjugation of maleimide in liposomes to the free thiols on ECFCs. The residual
439 maleimide groups on cell-bound particles were quenched with 1 mg/mL thiol-terminated 2-kDa
440 PEG for 30 mins in complete EGM-2 medium. After nanoparticles were conjugated on the cell
441 surface, their presence was confirmed by confocal microscopy (Nikon A1R-MP). Mean
442 fluorescence intensity (MFI) was measured using FACS (BD LSR Fortessa X-20) and FlowJo
443 software.

444 **In vitro Functional Assays**

445 Human ECFCs and GDM-ECFCs at passage 2-5 were assessed for their *in vitro*
446 function using cell migration, proliferation, wound healing, and vasculogenesis assays as
447 previously described.^{43,80,81} FACS, western blot, qRT-PCR were used to analyze protein and
448 gene expression levels. Details methods with information on primers and antibodies are
449 available in the **Supplementary Information**. Tube formation was quantified using the KAV
450 plugin in the FIJI program.^{30,82}

451

452 **In vivo Functional Assays**

453 Subcutaneous implantation of the cell-containing gel plugs was conducted with 8-week
454 old NOD-SCID mice following the procedure approved by Indiana University School of
455 Medicine IACUC.^{65,66} Briefly, mice were anesthetized by isoflurane. A small incision was made
456 to create two subcutaneous pockets near the dorsal flanks. One gel plug was inserted into each
457 pocket, one with Vh-NPs and one with SB-NPs. The incision was clipped and Ketoprofen
458 (100mg/ml) was injected for reducing pain. A perfusion study was performed on day 14 using
459 rhodamine-conjugated *Ulex Europaeus Agglutinin I* (UEA-I lectin) and fluorescein-conjugated
460 *isolectin Griffonia simplicifolia* (GS-IB4 isolectin).^{44,48} On day 14, the mice were euthanized and
461 the gel plugs were harvested and fixed with 4% paraformaldehyde. Tissue samples were
462 processed, sectioned and stained for H&E at the Histology and Histomorphometry Core,
463 Indiana Center for Musculoskeletal Health, IUSM. Sectioned slides were then stained for H&E
464 and IHC using human CD31 (clone JC70A, Dako), mouse CD31, and mouse SMA, as well as
465 appropriate IgG isotype controls (**Supplementary Fig. 11**).^{4,9} The number of human blood
466 vessels and size were counted, measured, and normalized to the graft area. We sampled a
467 minimum of 10 images for each graft, analyzed, and normalized the number and size of blood
468 vessels accordingly.

469 **Statistical Analysis**

470 Data are presented as mean $\pm$ standard deviation, unless otherwise were specified in
471 the figure legends. All statistical analysis were conducted in GraphPad Prism. Statistical
472 comparisons were made using Student's *t* test for paired data, analysis of variance (ANOVA)
473 for multiple comparisons, and with Tukey post hoc analysis for parametric data. Significance
474 levels were set at the following: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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492 and P.D.N conducted the experiments and analyzed the data. S.Z. and L.B. conducted the
493 perfusion study and intravital imaging. L.S.H and D.H.-P. supervised the study. All authors have
494 approved the manuscript.

495 **Data and materials availability:** Additional material and data which contributed to this study
496 are present in the **Supplementary Information**.

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715

716 **Figure 1. Synthesis and characterization of bioactive nanoparticles.** (A) Schematic of
717 maleimide-based conjugation of bioactive nanoparticles to the ECFC surface's free thiols
718 followed by *in situ* PEGylation. Continuous pseudo-autocrine stimulation of GDM-ECFCs with
719 SB-431542 improves their clinical potential for therapeutic angiogenesis. (B) A CryoTEM
720 photograph demonstrating the multilamellar structure of the nanoparticle. Scale bar is 50 nm.
721 (C-D) The stability of the nanoparticles was quantified using DLS for hydrodynamic diameter
722 (z-average) and polydispersity (PDI) over 30 days in buffer at (C) 4°C and (D) 37°C
723 (physiological temperature). The data represent the mean $\pm$ s.d. of three independent
724 experiments conducted in triplicate. (E) A confocal photograph demonstrating a stable
725 conjugation of Dil-labeled multilamellar lipid nanoparticles (red) conjugated onto the surface of
726 a CFSE-labeled ECFC (green). Scale bar is 10 μ m. (F) Flow cytometry analysis demonstrated
727 an increase in mean fluorescence intensity (MFI) when the ratio of cell to nanoparticles was
728 increased from 1:100 to 1:5,000. Inset indicates the corresponding histogram data. (G) The
729 viability of ECFCs with various cell to nanoparticle ratios (1:100 to 1:5,000) was quantified using
730 alamar blue assay over three days. No significant difference was observed between non-
731 conjugated cells and nanoparticle-conjugated cells (mean $\pm$ S.D., three independent
732 experiments conducted in triplicate). (H) Accumulative release of the bioactive small molecule,
733 SB-431542 (SB), from the NPs over 2 weeks. Three different initial concentrations of SB (10,
734 20, 40 μ M) were encapsulated into the NPs and the amounts of SB released were measured
735 daily. The release profiles were fit for Korsmeyer-Peppas equation and the fitting parameters
736 are shown within the graph.

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738 **Figure 2. Characterization of key ECFC phenotypes.** CFSE-labelled ECFCs were (A)
739 unmanipulated as control unconjugated cells or (B) conjugated with 5,000 Dil-labeled
740 multilamellar lipid nanoparticles per cell. Scatter plots demonstrate that nanoparticles remained

on the cell surface and split equally among daughter cells. Quantification of mean fluorescence intensity (MFI) of CFSE and Dil over 5 days for (C) control non-conjugated cells and (D) nanoparticle-conjugated cells (mean $\pm$ s.d., three independent experiments conducted in triplicate). Expression of cell surface markers (E) CD31, (F) CD34, and (G) CD144 were examined using flow cytometry for NP-conjugated cells (*green* lines) and compared to unconjugated cells (*grey* lines) and isotype controls (*yellow* lines). Nanoparticle-conjugated cells and their unconjugated counterparts expressed comparable levels of ECFC-specific markers, based on their MFIs quantified for (H) CD31, (I) CD34, and (J) CD144. Four biological replicates ($n=4$; mean $\pm$ s.d. of two independent experiments conducted in triplicate) were used for normal ECFCs (*black* data dots) and GDM-ECFCs (*red* data dots). Statistical significance was set at $^{\#}P>0.05$, $^{**}P<0.01$, $^{***}P<0.005$. Representative histograms from individual ECFC line can be found in **Supplementary Fig. 6**.

Figure 3. Treatment with SB-431542 reduces TAGLN expression in GDM-ECFCs. (A) Real-time RT-PCR quantification of *TAGLN* expression in normal ECFC and GDM-ECFC under Vehicle (DMSO) control or treatment with 5 μ M SB-431542 (SB) for 72 hr (three independent experiments, conducted in triplicate with 4 biological replicates per condition). (B) The inhibition effects of SB was transient, as the SB was removed from the medium at day 4, the *TAGLN* expression in GDM-ECFC at day 5 and 6 increased to level comparable to GDM-ECFC treated with vehicle control. (C) Real-time RT-PCR quantification of *TAGLN* expression in normal ECFC and GDM-ECFC conjugated with either Vh-NPs or SB-NPs after 6 days in culture (three independent experiments, conducted in triplicate with 4 biological replicates per condition). Bioactive SB-NPs provided a continuous down regulation of *TAGLN* at the mRNA level following 6 days in culture. (D) Representative Western blot evaluating TAGLN is shown

765 using whole cell lysates isolated at day 6 from normal ECFC (n=4) and GDM-ECFCs (n=4)
766 conjugated with either Vh-NPs or SB-NPs. Vinculin (VINC) is the loading control. (E) Band
767 intensities were quantified using Image J and TAGLN protein expression levels were
768 normalized using VINC. Data were analyzed by one-way ANOVA followed by Kruskal-Wallis
769 post-test. Statistical significance was set at $^{\#}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$.

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771 **Figure 4. Bioactive Nanoparticles Improve Cell Migration** (A) Transwell migration assays
772 were performed with normal ECFCs and GDM-ECFCs conjugated with Vh-NPs or SB-NPs.
773 Photomicrographs depict migrated ECFCs stained with crystal violet. Scale bar is 1mm. (B)
774 The number of migrating cells after 4 hr were quantified. Four biological replicates ($n=4$; mean
775 $\pm$ s.d. of three independent experiments conducted in triplicate) were used for normal ECFCs
776 (*black* data dots) and GDM-ECFCs (*red* data dots). SB-NPs significantly improve cell migration
777 of GDM-ECFCs ($^{*}P=0.039$), but not normal ECFCs ($^{\#}P=0.955$). (C) Wound healing assays
778 were performed with normal ECFCs and GDM-ECFCs treated with Vh-NPs or SB-NPs. High
779 contrast brightfield images depict migrated ECFCs at 0 hr and 14 hr post wound initiation. Scale
780 bar is 1mm. (D) Kinetic wound confluence curves indicate wound closure for normal ECFCs
781 and GDM-ECFCs treated with Vh-NPs or SB-NPs (mean $\pm$ s.d.). (E) Quantification of wound
782 confluence at 10 h post wound initiation. Four biological replicates ($n=4$; mean $\pm$ s.d. of three
783 independent experiments conducted in triplicate) were used for normal ECFCs (*black* data
784 dots) and GDM-ECFCs (*red* data dots). SB-NPs significantly improve wound closure of GDM-
785 ECFCs ($^{*}P=0.041$), but not normal ECFCs ($^{\#}P=0.988$). Statistical significance was evaluated
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787

788 **Figure 5. Bioactive Nanoparticles Improve *in vitro* Angiogenesis** (A) High contrast
789 brightfield images of ECFCs and GDM-ECFCs vascular tube formation on Matrigel 5 hr post
790 plating following treatment with Vh-NPs or SB-NPs. Scale bar is 1mm. (B) Kinetic analysis of
791 vasculogenesis (KAV) identifies closed networks formed over time. (C) The number of closed
792 networks 5 hr post-plating were quantified and plotted. SB-NPs significantly improve closed
793 networks formed by GDM-ECFCs ($**P=0.010$), but not by normal ECFCs ($\#P=0.218$). Four
794 biological replicates ($n=4$; mean $\pm$ s.d. of three independent experiments conducted in
795 triplicate) were used for normal ECFCs (*black* data dots) and GDM-ECFCs (*red* data dots). (D)
796 High contrast brightfield images of ECFCs and GDM-ECFCs forming 3D vascular networks in
797 collagen at 48 hr post encapsulation following conjugation with Vh-NPs or SB-NPs. High
798 magnification images of the dashed areas depict the vascular tube networks. Scale bars are
799 100 μ m. (E) Fluorescent images of ECFCs and GDM-ECFCs forming 3D vascular networks
800 were stained for F-actin (green) and nuclei (blue). Scale bars are 50 μ m. (F) The number of
801 closed networks 48 hr post encapsulation were quantified and plotted. Four biological
802 replicates ($n=4$; mean $\pm$ s.d. of three independent experiments conducted in triplicate) were
803 used for normal ECFCs (*black* data dots) and GDM-ECFCs (*red* data dots). SB-NPs
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806

807 **Figure 6. *In vivo* functionality of ECFCs conjugated with bioactive nanoparticles.** Normal
808 and GDM-ECFCs were conjugated with nanoparticles containing either vehicle (Vh) or 40 μ M
809 SB-431542 (SB). Conjugated ECFCs (100,000 cells/construct) were encapsulated in
810 collagen/fibronectin gels for 48 hr to form vascularized networks and then transplanted into the
811 side flanks of NOD/SCID mice ($n=4-6$ animals/group). Representative IHC images stained with

anti-human CD31 illustrate the graft harvested 14 days following transplantation (highlighted in white dotted circle) for **(A)** ECFCs (Vh-NP), **(B)** GDM-ECFCs (Vh-NP), and **(C)** GDM-ECFCs (SB-NP). Scale bars are 500 μm . Representative high magnification IHC images of grafts from **(D)** ECFCs (Vh-NP), **(E)** GDM-ECFCs (Vh-NP), and **(F)** GDM-ECFCs (SB-NP) after 14 days implantation into NOD/SCID mice stained with anti-human CD31 (brown). Arrows indicate human CD31⁺ vessels, which are perfused with murine erythrocytes. Scale bars are 30 μm . The number and size of human CD31⁺ vessels were quantified and plotted. Compared to the Vh-NPs control, SB-NPs conjugation increases **(G)** vessel density ($*P=0.011$) and **(H)** vessel area ($**P=0.0058$) formed by GDM-ECFCs *in vivo*. Five animals ($n=5$; mean $\pm$ s.d.) were used to evaluate each group: normal ECFCs (*black* data dots) and GDM-ECFCs (*red* data dots). **(I)** Representative intravital images of grafts pre-perfused with rhodamine-conjugated *UEA-I* lectin to stain the human vessels (in red) and fluorescein-conjugated *GS-IB4* to stain the mouse vessels (in green). 3D confocal rendering demonstrated the interaction between human vasculature (in red) and mouse vasculature (in green) for normal ECFC, GDM-ECFCs conjugated with Vh-NPs and GDM-ECFCs conjugated with SB-NPs. Scale bars are 40 μm . **(J)** Vessel quantification and analysis reveal the percent area covered by human vasculature (*UEA-I* lectin) and mouse vasculature (*GS-IB4* isolectin). SB-NPs conjugation to GDM-ECFCs results in the significant increase of both human vessels ($**P=0.021$) and mouse vessels ($**P=0.006$) that were interconnected to each other. Three animals ($n=3$; mean $\pm$ s.d.) were used to evaluate each group: normal ECFCs (*black* data dots) and GDM-ECFCs (*red* data dots). **(K)** Vessel quantification reveals the size distribution of the vessels found in the explant for normal ECFCs, GDM-ECFCs conjugated with Vh-NPs and GDM-ECFCs conjugated with SB-NPs. Compared to the Vh-NPs control, SB-NPs conjugation results in an increase in the mean of vessel size distribution from for GDM-ECFCs ($**P=0.0018$).

836 **Figure 7. Schematic representation of the cell surface conjugation strategy and**
837 **functionality study.** Children born from mothers with GDM experience increased risk of
838 developing T2DM, hypertension, and cardiovascular disease later in life. Key to this enhanced
839 risk is stress-induced dysfunction of vascular progenitor cells, including ECFCs. Cord blood-
840 derived ECFCs isolated from patients with GDM overexpress TAGLN, demonstrate reduced
841 cell migration, and exhibit impaired tube formation compared to normal ECFCs. Cell surface
842 conjugation with bioactive nanoparticles enable pseudo-autocrine stimulation of GDM-ECFCs
843 with SB-431542. Rejuvenation of GDM-ECFCs normalizes TAGLN expression, preserves key
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Figures

Figure 1

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Synthesis and characterization of bioactive nanoparticles. (A) Schematic of maleimide-based conjugation of bioactive nanoparticles to the ECFC surface's free thiols followed by in situ PEGylation. Continuous pseudo-autocrine stimulation of GDM-ECFCs with SB-431542 improves their clinical potential for therapeutic angiogenesis. (B) A CryoTEM photograph demonstrating the multilamellar structure of the nanoparticle. Scale bar is 50 nm. (C-D) The stability of the nanoparticles was quantified using DLS for hydrodynamic diameter (z-average) and polydispersity (PDI) over 30 days in buffer at (C) 4°C and (D)

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Characterization of key ECFC phenotypes. CFSE-labelled ECFCs were (A) unmanipulated as control unconjugated cells or (B) conjugated with 5,000 Dil-labeled multilamellar lipid nanoparticles per cell. Scatter plots demonstrate that nanoparticles remained on the cell surface and split equally among daughter cells. Quantification of mean fluorescence intensity (MFI) of CFSE and Dil over 5 days for (C) control non-conjugated cells and (D) nanoparticle-conjugated cells (mean $\pm$ s.d., three independent

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Schematic representation of the cell surface conjugation strategy and functionality study. Children born from mothers with GDM experience increased risk of developing T2DM, hypertension, and cardiovascular disease later in life. Key to this enhanced risk is stress-induced dysfunction of vascular progenitor cells, including ECFCs. Cord blood-derived ECFCs isolated from patients with GDM overexpress TAGLN, demonstrate reduced cell migration, and exhibit impaired tube formation compared to normal ECFCs. Cell surface conjugation with bioactive nanoparticles enable pseudo-autocrine stimulation of GDM-ECFCs with SB-431542. Rejuvenation of GDM-ECFCs normalizes TAGLN expression, preserves key progenitor phenotypes, improves cell migration and tube formation in vitro, as well as augments the density and area of chimeric vessels formed in vivo. This cell surface conjugation strategy to rejuvenate GDM-ECFCs and improve their clinical potentials can be used for therapeutic angiogenesis to address various complications precipitated from GDM.

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