

Role of Platelets in Placentation

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Running title: Platelet and Placentation

Key words: chemokine / extravillous trophoblast / endovascular trophoblast / preeclampsia /
intrauterine fetal growth restriction / vascular remodeling /

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Abstract

In human placenta, embryo-derived trophoblasts aggressively invade maternal spiral arteries and transform the arteries to low-resistant large-caliber vessels. This process that ensures adequate placental perfusion is called maternal vascular remodeling. Histological examination showed deposition of maternal platelets in the trophoblast aggregates formed in the spiral arteries. Several lines of evidence suggest that these platelets are activated. Soluble factors released from the activated platelets, as whole, enhanced invasive capacity of isolated trophoblasts in vitro. These findings suggest importance of non-hemostatic platelet function in maternal vascular remodeling. In contrast, gene knockout studies suggest that maternal platelet defects are compatible with successful pregnancy in mice. Moreover, pregnant women with severe platelet defects usually fulfill uneventful pregnancy. Thus, promotion of endovascular trophoblast infiltration by maternal platelets might not be the only mechanism that regulates maternal vascular remodeling.

The maternal vascular remodeling is an essential component of human reproduction and should be secured by several complementary mechanisms. Future studies should aim to elucidate other mechanisms that could regulate endovascular trophoblast infiltration.

1 Introduction

2
3 In human placenta, cytotrophoblasts show two distinct patterns of differentiation (Figure 1).
4 In floating villi, cytotrophoblasts differentiate into syncytiotrophoblast and form the syncytial
5 layer, where exchange of gas and nutrients takes place. On the other hand, at
6 villus-anchoring sites, cytotrophoblasts differentiate into extravillous trophoblasts and form
7 the stratified structure called cell column. After losing proliferative activity and acquiring
8 invasive activity in the cell column¹, extravillous trophoblasts begin to invade decidual tissue
9 (interstitial trophoblasts) or maternal blood vessels (endovascular trophoblasts).

10 Endovascular trophoblasts destroy the muscular linings and replace the endothelium of the
11 maternal spiral arteries, transforming them from small resistant vessels to flaccid large-caliber
12 vessels. This process is called maternal vascular remodeling. The maternal vascular
13 remodeling ensures adequate placental perfusion and contributes to establishment of
14 successful pregnancy². In fact, endovascular trophoblast invasion is limited to superficial
15 decidua and the myometrial segments remain narrow in cases of preeclampsia and/or
16 intrauterine growth restriction³.

17 Vascular infiltration by endovascular trophoblast is a unique phenomenon in primate
18 placenta⁴. Moreover, in most of non-human primates, vascular remodeling is restricted to
19 the decidua, which in human has to be considered as pathological. Lack of proper
20 experimental model animal has hampered analysis of endovascular trophoblast invasion and
21 the mechanism is still largely unknown.

Platelet in human placentation

What directs extravillous trophoblasts towards the maternal spiral arteries? It is intuitive to consider that some factor(s) derived from the endothelium or blood constituents direct this movement. Relatively high oxygen tension in maternal arteries promotes trophoblast differentiation toward invasive phenotype, which could be one of the mechanisms that facilitate endovascular trophoblast invasion⁵. This hypothesis is derived from the findings that isolated cytotrophoblasts cultured under hypoxic conditions (2% O₂) continued proliferating, whereas those cultured in 20% O₂ stopped proliferating and differentiated to invasive phenotype. Another candidate that facilitates endovascular trophoblast invasion could be maternal platelets⁶. Histological examination of human placental bed revealed that maternal platelets are trapped by endovascular trophoblast aggregates that are formed inside the lumen of the spiral arteries. These platelets were attached to collagen deposited around endovascular trophoblasts, suggesting that these platelets are activated. Indeed, these platelets expressed P-selectin. P-selectin is one of activation markers of platelets that binds to P-selectin glycoprotein ligand-1 (PSGL-1) on the surface of neutrophils, leading to recruitment and activation of the neutrophils. The activated neutrophils, in turn, release platelet-activating factor (PAF) that promotes platelet aggregation⁷. In vitro, co-culturing with platelets that were activated by collagen induced matrigel invasion of extravillous trophoblasts isolated from early human placental tissue. After 48-hour culture, most of the isolated extravillous trophoblasts exhibit elongated spindle-shaped morphology mimicking interstitial trophoblasts, whereas they are transformed to round-shaped morphology mimicking endovascular trophoblasts after 48-hour co-culture with platelets (Figure 2). The round-shaped cells share similar molecular property with endovascular trophoblasts in vivo,

such as up-regulation of integrin $\alpha 1$ and CCR1^{8 9}. These changes do not require direct contact of platelets and isolated extravillous trophoblasts, suggesting that some soluble factor(s) derived from the activated maternal platelets direct trophoblast invasion towards maternal arteries and induce differentiation towards endovascular phenotype.

Then, what are the platelet-derived soluble factors that enhance trophoblast invasion? Besides regulating hemostasis, platelets contain a number of bioactive peptides, including growth factors (EGF, VEGF, PDGF-B, TGF- $\beta 1$, IGF-1, HGF, bFGF), cytokines (soluble CD40 ligand), chemokines (RANTES, β TG, PF4), and bioactive phospholipids (S1P). These mediators are stored in platelet granules and released upon stimulation¹⁰. The effect of platelet-conditioned medium to promote invasion of the isolated extravillous trophoblasts was completely abrogated by heat treatment, but not by charcoal stripping⁹. This suggests that some growth factors, cytokines, or chemokines, but not bioactive phospholipids contribute to the invasion-promoting effect of platelets. Immunocytochemistry revealed that isolated extravillous trophoblasts express a chemokine receptor, CCR1. Matrigel invasion assay revealed that RANTES, one of CCR1 ligands, enhances invasion of isolated extravillous trophoblasts, indicating that CCR1 expressed on isolated extravillous trophoblasts is a functional receptor. Interestingly, immunohistochemistry of the placental bed showed that CCR1 is predominantly expressed from the cell column through endovascular trophoblasts. These findings suggest that circulating maternal platelets are trapped by endovascular trophoblast aggregates and activated to release CCR1 ligands, which in turn attracts CCR1-positive extravillous trophoblasts into maternal spiral arteries to encourage the vascular remodeling (Figure 3).

In this theory, activation of platelets in the spiral arteries requires the preexistence of endovascular trophoblast aggregates. In this respect, platelet is not a primary initiator of

- 1 extravillous trophoblast invasion into the spiral arteries; rather it might provide a positive
- 2 feedback mechanism that facilitates endovascular trophoblast infiltration.
- 3

Platelet in mouse placentation

Gene knockout has generated several mice lineages that have quantitative or qualitative platelet defect. The transcription factor NF-E2 is essential for megakaryopoiesis and NF-E2-deficient mice have severe quantitative platelet defect^{11 12}. NF-E2-null murine embryos are able to complete intrauterine life, although they show significant growth restriction at birth. The growth restriction is associated with abnormal vascularization of the labyrinthine layer in NF-E2-null placenta. Since the number of blood vessels in the labyrinthine layer is not reduced, abnormal vascularization is considered to be due to failure in the maturation of preexisting vessels. These indicate that embryonic platelets are required for normal placentation¹³.

In mice lacking α -subunit of the heterotrimeric guanine nucleotide binding protein Gq ($G\alpha_q$), platelet count is normal but these platelets cannot be activated in vitro with physiological agonists including thrombin, ADP, and collagen¹⁴. In contrast to NF-E2 null mice, vascularization of $G\alpha_q$ -null placenta is not affected, suggesting that hemostatic platelet function is not required for normal placentation. In other words, non-hemostatic function of platelets, which is only revealed in mice with quantitative platelet defect, is essential for the maturation of the fetus-derived vessels in the placenta¹³.

Both quantitatively and qualitatively platelet-deficient mice are able to complete intrauterine development and some of them even survive to reproductive age. These adult female mice allow analysis of possible effects of maternal platelet defect on placentation. In contrast to NF-E2 null embryos, the litter size of NF-E2 null mothers is not reduced as long as embryo has normal platelet count. Detailed analysis of the placental histology showed that massive placental hemorrhage occurs in approximately half of the placentas of NF-E2 null

1 mothers most probably due to maternal bleeding diathesis ¹⁵. Thus, maternal platelet is not
2 required for normal murine placentation.

3

4

Platelet and human pathological pregnancies

Primary hemostasis begins immediately after endothelial disruption. The exposed subendothelial collagen recruits circulating von-Willebrand factor, which binds to platelet surface glycoprotein Ib-IX-V complex and thus mediates the contact between collagen and platelets. Collagen-activated platelets form pseudopods that stretch out to cover the injured surface and express receptors for fibrinogen that mediates primary platelet aggregation. Next, bioactive substances such as thromboxane A₂ are released from the activated platelets (platelet degranulation) and further activate surrounding platelets. These activated platelets express glycoprotein IIb-IIIa complex that can bind to von-Willebrand factor. Secondary platelet aggregation is mediated by interaction between von-Willebrand factor and glycoprotein IIb-IIIa complex, leading to primary thrombus formation. In the secondary hemostasis, coagulation cascade efficiently progresses on the phospholipid exposed on the surface of the aggregated platelets to solidify the primary thrombus (secondary thrombus formation).

Low-dose aspirin has been used worldwide to prevent placenta-mediated obstetric complications such as recurrent pregnancy loss, intrauterine growth restriction, and preeclampsia¹⁶. Aspirin inhibits platelet cyclooxygenase to suppress synthesis of thromboxane A₂ that induces secondary platelet aggregation and systemic vasoconstriction. Thus, theoretically, aspirin can inhibit thrombus formation in the placenta to help maintain placental blood flow as well as ameliorate hypertension in preeclampsia. In fact, recent meta-analysis established moderate but consistent preventative effect of low-dose aspirin therapy on preeclampsia¹⁷. Since aspirin does not interfere with platelet adhesion to collagen or subsequent platelet degranulation, platelet release of bioactive soluble factors

1 other than thromboxane A₂ should be maintained during low-dose aspirin therapy.
2 Therefore, in terms of maternal vascular remodeling, aspirin does no harm to endovascular
3 trophoblast invasion enhanced by platelet-derived soluble factors.

4 Secondary thrombus formation generally ensues after platelet activation. Given that
5 maternal platelets trapped by the endovascular trophoblasts are activated, it seems peculiar
6 that no fibrin deposition is detected among the endovascular trophoblast aggregates.
7 Although the actual mechanism for this discrepancy remains to be elucidated, abundant
8 thrombomodulin¹⁸ and tissue- and urokinase-type plasminogen activators^{19 20 21} expressed by
9 trophoblasts could inhibit the coagulation cascade and/or facilitate rapid degradation of the
10 fibrin at the surface of the endovascular trophoblasts. In pathological pregnancies such as
11 preeclampsia, it is fascinating to consider that inhibitory mechanism against the secondary
12 thrombus formation around the endovascular trophoblasts is defective, thus leading to
13 excessive placental thrombosis. In this situation, aspirin could be particularly beneficial,
14 because it can inhibit platelet secondary aggregation that triggers the initiation of the
15 coagulation cascade without affecting endovascular trophoblast invasion.

16 Bernard-Soulier syndrome is characterized by deficiency of platelet glycoprotein Ib-IX-V
17 complex that mediates contact of platelets with collagen²². In this pathological condition,
18 collagen-activation of platelets is severely impaired and most of the patients exhibit
19 spontaneous bruising, epistaxis, and bleeding after minor trauma. In pregnancy, entrapment
20 of maternal platelets by collagen-deposited endovascular trophoblasts and the subsequent
21 activation should be restricted, resulting in insufficient maternal vascular remodeling.
22 However, pregnant women with Bernard-Soulier syndrome as well as those with congenital
23 thrombocytopenia generally complete uneventful pregnancy despite an increased risk of
24 postpartum hemorrhage²³⁻²⁵. This suggests that maternal platelets are not an essential

- 1 component of human placentation process including maternal vascular remodeling.
- 2 Although in vitro data suggest importance of maternal platelets in endovascular trophoblast
- 3 infiltration, their actual role in vivo still needs to be defined.
- 4

Conclusion and future direction

In human placenta, maternal platelets are deposited in trophoblast clusters that have invaded the uterine spiral arteries. These platelets are likely to be activated and release various soluble factors, which as whole enhance invasive capacity of extravillous trophoblasts in vitro. Thus, maternal platelets might be a candidate that attracts extravillous trophoblasts into the spiral arteries and encourages maternal vascular remodeling. As demonstrated in gene-knockout mice with quantitative or qualitative platelet defects, however, maternal platelets are not required for murine placentation. The fact that pregnant women with severe platelet defects are compatible with uneventful pregnancy suggests that maternal platelets are not an essential component of human placentation.

The maternal vascular remodeling that ensures adequate placental perfusion is an essential component of human reproduction and should be secured by several complementary mechanisms. In this respect, promotion of endovascular trophoblast infiltration by maternal platelets could be one of the mechanisms that regulate the vascular remodeling, although the vascular remodeling might occur in the absence of maternal platelets.

Precise characterization of endovascular trophoblasts, which is a crucial step for clarification of the mechanism(s) of maternal vascular remodeling, has been hampered by the extreme difficulty in their isolation from human placenta. As mentioned above, co-culture with platelets could induce differentiation of isolated extravillous trophoblasts towards endovascular phenotype in vitro. These endovascular-like trophoblasts are potentially useful to characterize endovascular trophoblasts, leading to identification of novel mechanisms of maternal vascular remodeling.

References

- [1] Damsky CH, Fitzgerald ML, Fisher SJ. Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vivo. *J Clin Invest.* 1992; **89**(1): 210-22.
- [2] Ramsey EM. The story of the spiral arteries. *J Reprod Med.* 1981; **26**(8): 393-9.
- [3] Khong TY, De WF, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol.* 1986; **93**(10): 1049-59.
- [4] Frank HG, Kaufmann P. Nonvillous parts and trophoblast invasion. In: Benirschke K, Kaufmann P, (eds) *Pathology of Human Placenta*. New York: Springer 2000; 171-272.
- [5] Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. *Science.* 1997; **277**(5332): 1669-72.
- [6] Sato Y, Fujiwara H, Zeng BX, Higuchi T, Yoshioka S, Fujii S. Platelet-derived soluble factors induce human extravillous trophoblast migration and differentiation: platelets are a possible regulator of trophoblast infiltration into maternal spiral arteries. *Blood.* 2005; **106**(2): 428-35.
- [7] Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev.* 2007; **21**(2): 99-111.
- [8] Zhou Y, Genbacev O, Damsky CH, Fisher SJ. Oxygen regulates human cytotrophoblast differentiation and invasion: implications for endovascular invasion in normal pregnancy and in pre-eclampsia. *J Reprod Immunol.* 1998; **39**(1-2): 197-213.

- 1 [9] Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S. Trophoblasts
2 acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype.
3 *Development*. 2003; **130**(22): 5519-32.
- 4 [10] Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their
5 relevance to platelet storage and transfusion practice? *Transfus Med*. 2001; **11**(6): 403-17.
- 6 [11] Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, *et al*. Transcription factor
7 NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF
8 in megakaryocyte development. *Cell*. 1995; **81**(5): 695-704.
- 9 [12] Levin J, Peng JP, Baker GR, *et al*. Pathophysiology of thrombocytopenia and
10 anemia in mice lacking transcription factor NF-E2. *Blood*. 1999; **94**(9): 3037-47.
- 11 [13] Palumbo JS, Zogg M, Talmage KE, Degen JL, Weiler H, Isermann BH. Role of
12 fibrinogen- and platelet-mediated hemostasis in mouse embryogenesis and reproduction. *J*
13 *Thromb Haemost*. 2004; **2**(8): 1368-79.
- 14 [14] Offermanns S, Toombs CF, Hu YH, Simon MI. Defective platelet activation in G
15 alpha(q)-deficient mice. *Nature*. 1997; **389**(6647): 183-6.
- 16 [15] Isermann B, Nawroth PP. The role of platelets during reproduction. *Pathophysiol*
17 *Haemost Thromb*. 2006; **35**(1-2): 23-7.
- 18 [16] James AH, Brancazio LR, Price T. Aspirin and reproductive outcomes. *Obstet*
19 *Gynecol Surv*. 2008; **63**(1): 49-57.
- 20 [17] Askie LM, Duley L, Henderson-Smart DJ, Stewart LA, Group PC. Antiplatelet
21 agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet*.
22 2007; **369**(9575): 1791-8.
- 23 [18] Isermann B, Sood R, Pawlinski R, *et al*. The thrombomodulin-protein C system is
24 essential for the maintenance of pregnancy. *Nat Med*. 2003; **9**(3): 331-7.

- [19] Strickland S, Richards WG. Invasion of the trophoblasts. *Cell*. 1992; **71**(3): 355-7.
- [20] Teesalu T, Blasi F, Talarico D. Expression and function of the urokinase type plasminogen activator during mouse hemochorial placental development. *Dev Dyn*. 1998; **213**(1): 27-38.
- [21] Hu ZY, Liu YX, Liu K, *et al*. Expression of tissue type and urokinase type plasminogen activators as well as plasminogen activator inhibitor type-1 and type-2 in human and rhesus monkey placenta. *J Anat*. 1999: 183-95.
- [22] Nurden AT, Nurden P. Inherited defects of platelet function. *Rev Clin Exp Hematol*. 2001; **5**(4): 314-34; quiz following 431.
- [23] Khalil A, Seoud M, Tannous R, Usta I, Shamseddine A. Bernard-Soulier syndrome in pregnancy: case report and review of the literature. *Clin Lab Haematol*. 1998; **20**(2): 125-8.
- [24] Turner RJ, Spencer RA, Miyazawa K. Successful cesarean section in a gravida with the thrombocytopenia with absent radius syndrome. A case report. *J Reprod Med*. 1986; **31**(4): 260-2.
- [25] Peitsidis P, Datta T, Pafilis I, Otomewo O, Tuddenham EG, Kadir RA. Bernard Soulier syndrome in pregnancy: a systematic review. *Haemophilia*. 2010.

Figure legend

Figure 1 Schematic representation of early human placenta.

In floating villus, cytotrophoblasts (light gray cells) differentiate into multinucleated syncytiotrophoblast (dark gray cells) and form the syncytial layer, where exchange of gas and nutrients takes place. At villus-anchoring sites, cytotrophoblasts differentiate into extravillous trophoblasts (spotted cells) and form the stratified structure called cell column. Extravillous trophoblasts acquire invasive activity in the cell column and begin to invade the decidual tissue (interstitial trophoblasts) or the spiral arteries (endovascular trophoblasts).

Figure 2 Platelet-derived soluble factors induce endovascular differentiation of isolated human extravillous trophoblasts.

Isolated human extravillous trophoblasts were cultured for 48 hours in the absence (A, control) or in the presence of human platelets (B, platelet co-culture) that were plated in the collagen type I-coated upper chamber. Note that most of extravillous trophoblasts exhibit round-shaped morphology (arrows) mimicking endovascular trophoblasts after platelet co-culture (B).

Figure 3 An illustration showing possible chemokine gradient produced by platelets in spiral arteries and its estimated effects on endovascular trophoblast infiltration.

In the spiral artery undergoing vascular remodeling, platelets are deposited among the endovascular trophoblasts. These platelets are likely to have been activated by extracellular matrix secreted from endovascular trophoblasts and to have released various soluble factors including CCR1 ligands, forming local chemokine gradient. The chemokine gradient directs

- 1 CCR1-positive extravillous trophoblasts into the spiral artery, providing a positive feedback
- 2 cascade for trophoblastic arterial infiltration. Note that CCR1 is expressed from the cell
- 3 column through endovascular trophoblasts.

Figure 1

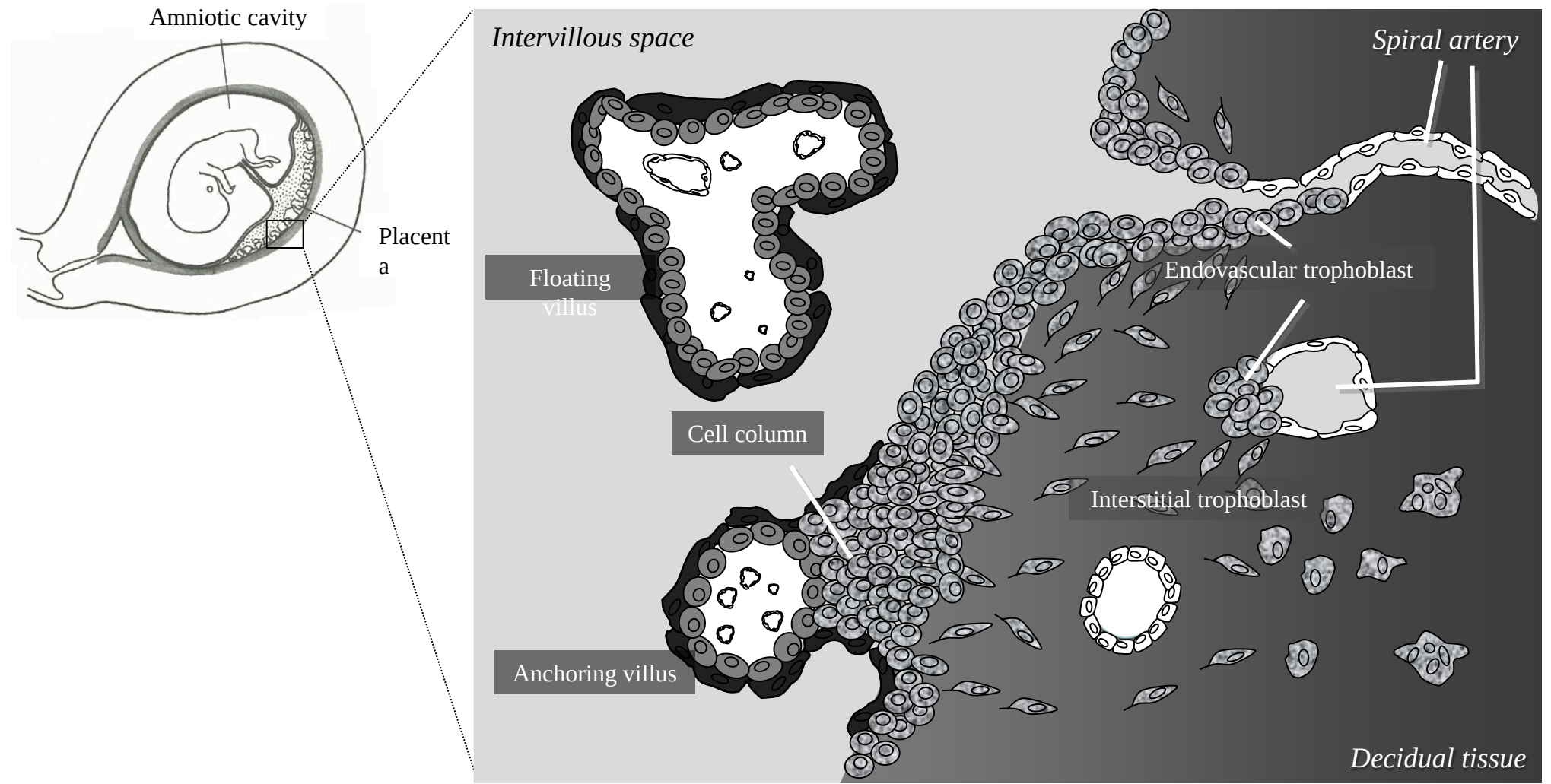


Figure 2

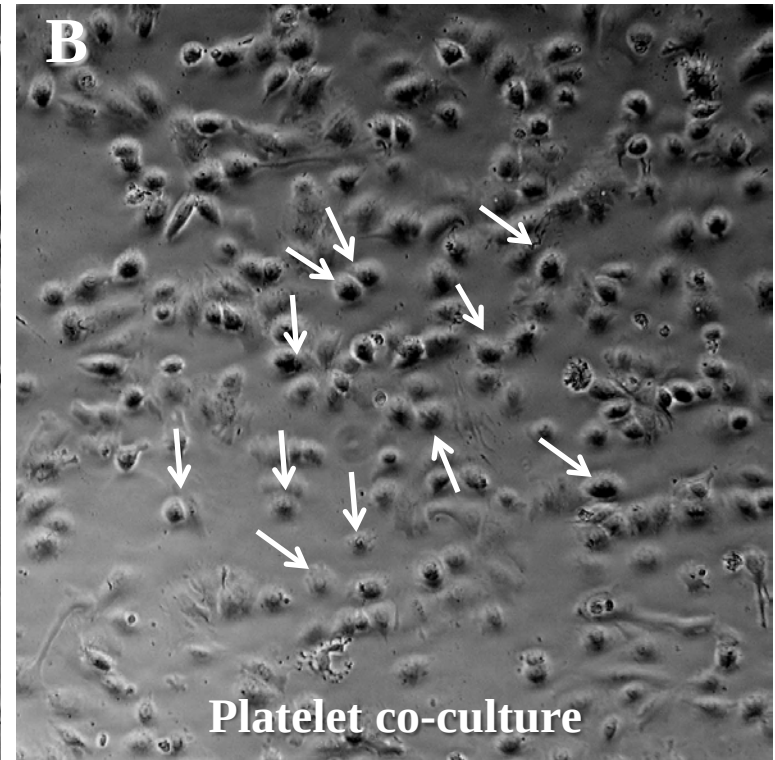
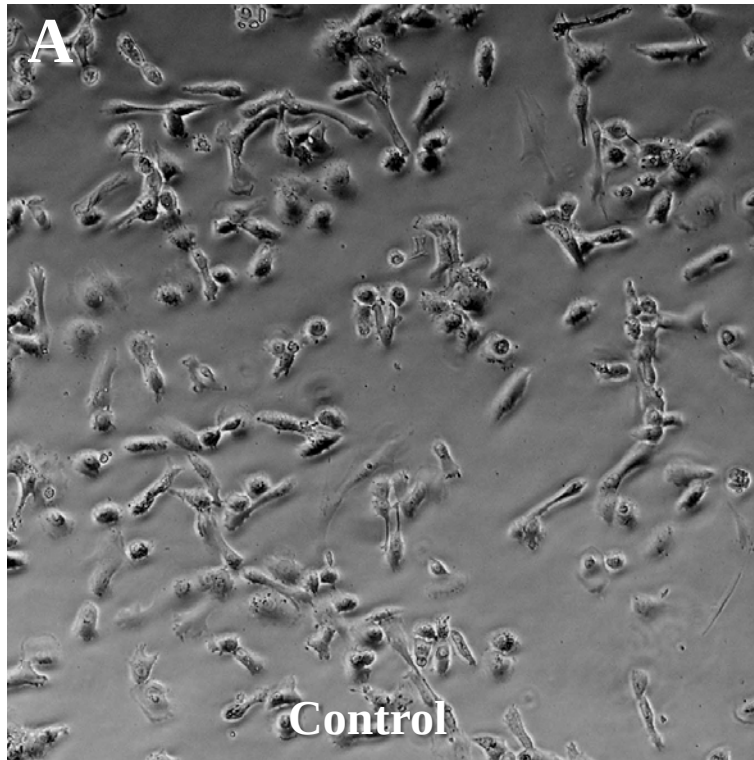


Figure 3

