1   **Unveiling mesenchymal stromal cells' organizing function in regeneration**

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Abstract

Regeneration is a fundamental process much attributed to functions of adult stem cells. In last decades delivery of suspended adult stem cells is widely adopted in regenerative medicine as a leading mean of cell therapy. However, adult stem cells can not complete the task of human body regeneration effectively by themselves as far as they need a receptive microenvironment (the niche) to engraft and perform properly. Understanding of mechanisms underlying mammalian regeneration lead us to an assumption that improved outcomes of cell therapy requires a specific microenvironment generated in damaged area prior to stem cell delivery. To certain extent it may be achieved by delivery of mesenchymal stromal cells (MSC), not in dispersed form, but rather self-organized in cell sheets (CS) – tissue-like structures comprising of viable cells and microenvironment components: extracellular matrix and soluble factors deposited in the matrix.

In this communication we highlight a potential role of mesenchymal stromal cells (MSC) as regeneration organizers and speculate that this function emerges in CS. This concept shifts our understanding of therapeutic mechanism underlying a widely known CS-based delivery method for regenerative medicine.

Keywords: stem cell, stromal cell, mesenchymal stromal cell, regeneration, cell sheet, cell delivery

1. The challenge for stem cell therapy: importance of microenvironment

In last decades stem cell therapy has moved from use of fetal and embryonic material to adult stem cell application pursuing the goal of damaged tissue structural and functional restoration [1]. Adult stem cells localized in majority of organs driving their physiological renewal and recovery after damage or disease [2]. This natural mechanism of human body regeneration provided a promising tool for regenerative medicine and resolved ethical problems related to fetal material use as well as major safety concerns connected with use of undifferentiated and/or potentially teratogenic cells [3, 4].

Despite initial promise of stem cell therapy it showed limited efficacy in numerous clinical trials [5] and, thus, put to doubt initial concept portraying adult stem cells as regenerative "commando engineers" that can engraft, differentiate and rebuild fully functional tissue once delivered viable and in sufficient amounts [6]. Eventually, accumulated data led to a conclusion that most of delivered stem cells fail to integrate and give rise to new tissue elements and work as "bystanders" releasing paracrine factors and disappearing within several weeks [7-9].

Discussing reason of disappointing results of stem cell therapy we may question what can be improved to overcome these hurdles in the way of the promising approaches in biomedicine. To answer that crucial role of microenvironment should be considered – from location of cell to set of signals required to maintain finely tuned physiological functions [10]. This issue is known since Raymond Schofield's seminal work suggesting that stem cells are "non-autonomous" and depend on microenvironment known as *niche* [11]. The latter controls, directs and supports balanced stages of stem cell life cycle and includes other cells (stromal, parenchymal, vascular etc.), extracellular matrix (ECM), soluble proteins and peptides, extracellular vesicles, small molecules and chemical factors (pH, oxygen pressure etc.) [12].

Importance of microenvironment for success of stem cell-driven body repair has been supported by investigation of regeneration in animals with ability to rebuild body parts or even whole organism after injury (e.g. planarians, starfish, axolotl). In

71   these creatures "separated" adult stem cells (neoblasts) themselves lack ability to
72   drive full-scale regeneration and require certain amount of tissues to preform
73   properly [13].

74   Human adult stem cells administered to damage site in suspension are
75   deprived of crucial stimuli required for their regenerative potential to unfold. They
76   lack appropriate intercellular contacts, nutrition and regulatory signals. Under such
77   unfavorable conditions formation of mature tissue elements from transplanted stem
78   cells is an extremely rare event hardly providing significant therapeutic impact [14,
79   15].

80   In this paper we summarize our vision on problems that stand in the way of
81   successful application of stem cell therapies. We focus on a cornerstone role of
82   microenvironment formed during regeneration and contribution of mesenchymal
83   stromal cells (MSC) to this process. We suggest that tissue engineered constructs
84   known as cell sheets (CS) present a feasible tool to unfold potential of MSC as
85   organizers of regeneration.

86

2. Shifting the focus to microenvironment: feeder needed!

88   To illustrate the possible reason of stem cell therapies failure and support our
89   accent on importance of receptive microenvironment for success of therapeutic
90   approach we shall start with a metaphor to compare mammalian tissue regeneration
91   with repopulation of an ecosystem after a natural catastrophe.

92   It is well-known that structure of ecological systems has hierarchy based on
93   food chains. It is typically portrayed as a pyramid reflecting dependency of high-
94   order consumers on lower-order and down to the ground level of energy-absorbing
95   producers – plants and microorganisms (left part of Figure 1).

96   After disaster hierarchy of ecosystem and its structure recovers stage by stage
97   from the foundation starting with the most adaptive and viable species - producers.
98   When they generate a necessary trophic substrate then more demanding species can
99   enter this system and populate it. Gradually the species diversity of entire ecosystem
100 increases and populations begin to interact controlling each other until hierarchy of

101   ecosystem is restored and recovery occurs [16]. However, the cornerstone of this

102   process is – and it should be emphasized – formation of a "ground level" of

103   producers required for other species to survive [17].

104   This metaphor was used to show why therapies using stem cells may fail. In

105   acute phase of response to injury we deliver them to a microenvironment lacking

106   necessary elements and regulatory framework that existed before damage. We

107   speculate that stages of ecosystem recovery might portray regeneration in humans

108   and illustrate that stem cells are capable to unveil their potential only when adequate

109   microenvironment is generated prior to that. Indeed, after damaged area is cleansed

110   by inflammatory cells, they leave "ground zero" with disrupted structure of the

111   tissues down to molecular level. Under such conditions stem cell fails to rebuild the

112   tissue as it depends on other elements – ECM, soluble factors, endothelium, stromal

113   cells and neural terminals disrupted by damage [10-12].

114   In ecological terms this may be described as a high-order consumer entering

115   the vast field of ashes after a forest fire before the lower levels are re-populated.

116   Failing to find food and mate eventually he will be leaving the unsuitable habitat.

117   Following this idea basal layer of right side of the pyramid - the *feeder* becomes of

118   paramount importance for microenvironment rebuilding to involve specific

119   progenitors (right part of Figure 1) similarly to the ecological dogma that producers

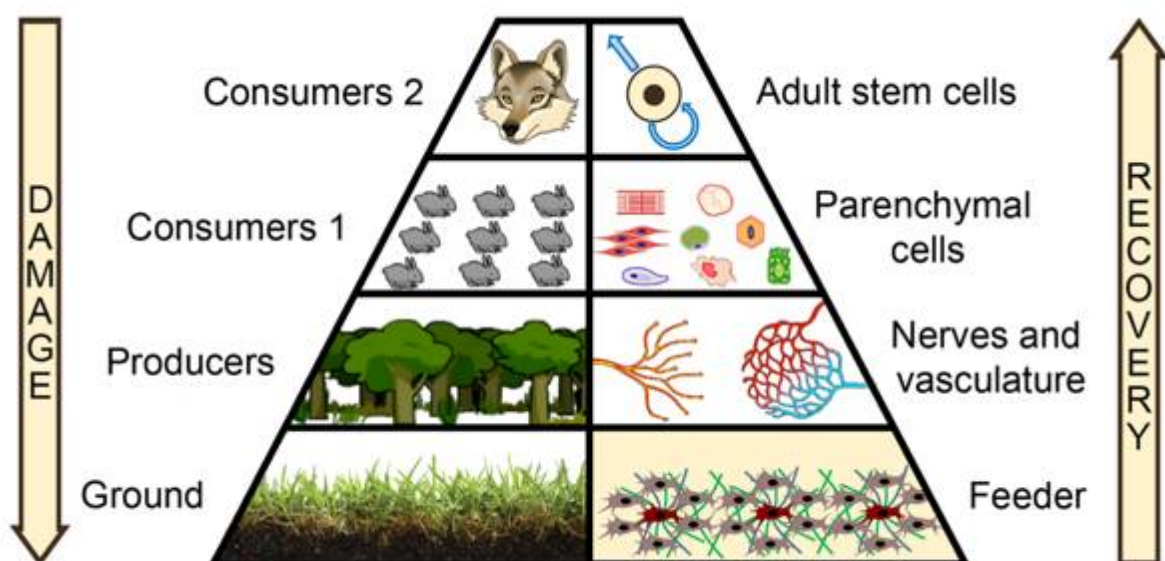120   presence is obligatory prior to consumers' population appears.

121

122   **Figure 1. Critical role of feeder in sequential regeneration of human**
123   **body.**

124   Both in ecosystem and human body "ground" and "feeder" levels respectively
125   are generated by the most adaptive and universal inhabitants. They have critical
126   importance as they provide foundation for subsequent interactions between elements
127   and the system structure recovery (see text for detail).

128

129   We define the *feeder* as a transient heterogenous structure formed *in vivo* at
130   early stage of regeneration to re-create disrupted components of microenvironment
131   which allows other cell types to perform properly in recovery of structure and
132   function. In our concept this putative feeder may contain stromal, immune and
133   probably vascular cells and intercellular components of microenvironment: ECM
134   and soluble factors predominantly anchored in the matrix to render paracrine effects
135   and create gradients of stimuli mapping further stages of regeneration.

136   Similar requirement for feeder has been described in zoology prior to
137   formation of blastema after amputation of a limb in salamander. Immediately after
138   injury a layer of epidermal cells covers the wound and becomes an *apical epithelial*
139   *cap* providing ground for attraction of fibroblasts and other cells that dedifferentiate
140   to form the blastema and eventually regrow the limb [18].

141   An encouraging example of whole organ recapitulation from delivered stem
142   cells in humans is bone marrow transplantation [19]. However, in this procedure
143   after myeloablative stage the stromal component of bone marrow remains relatively
144   intact providing a functional niche for transplanted hematopoietic cells supporting
145   the idea that receptive microenvironment is critical for regeneration allowing stem
146   cells to rebuild tissue but not work as secretome-producing "bystanders" [14, 15].

147   Typical example of a non-receptive microenvironment that can be formed
148   after damage is scar – avascular bulk of ECM packed to hold the tissues together
149   and barely undergoing resolution over time. Stem cell therapies have shown little to
150   no effect in stimulation of fibrosis resolution yet in some disorders like cystic
151   fibrosis or idiopathic pulmonary fibrosis they have been shown to attenuate disease

152 progress [20]. Nevertheless, these effects were once again generally attributed to
153 cells' paracrine function rather than engraftment and tissue restoration [8].

154

155   ### 3. Re-building the feeder: focus on mesenchymal stromal cells (MSC)

156   Thus, we come to a conclusion that stem cell therapies do not achieve their
157 goal until a receptive microenvironment is being formed. Due to a defined order of
158 events during regeneration after injury we suggest to focus not only on tissue-
159 specific stem cells but on the basal layer for which we provide the term *feede*r (in
160 particular, similarly to cell culture technique used to support stem cells and other
161 cell types). Below we speculate on participation of MSC in its formation *in vivo* and
162 address their biological and physiological functions that support this point.

163   Among numerous cell types of human body MSC remain a prominent
164 candidate for therapeutic use and in our concept they comprise an important (yet not
165 the only) component of putative feeder. In human body MSC are involved in
166 maintaining of structure and homeostasis [21] comprising an ubiquitous stromal
167 component in majority of organs. In quiescent state they are localized in perivascular
168 compartments supporting vascular permeability, secrete growth factors, support
169 ECM production and turnover. These functions are important for interaction with
170 local tissue progenitors and stem cells to retain organs architecture by maintaining
171 gradients of stimuli. In intestinal crypt niche MSC create a gradient of bone
172 morphogenic proteins (BMP) that controls resident stem cell fate and differentiation
173 [22]. However, this tissue function of MSC is not limited to stem cell niches and in
174 intact organs they participate in formation of microenvironment for all cells located
175 nearby [23].

176   After damage MSC migrate to the lesion site where they actively proliferate
177 [24]. Their ability to survive under stress resulted in excellent cultural properties of
178 isolated MSC. Upon cease of acute phase of inflammation MSC attenuate intensity
179 of residual inflammation. They secrete a wide range of anti-inflammatory factors
180 (IL-10, TSG-6, PGE_2) and, thus, switch the microenvironment to a pro-regenerative
181 state reducing oxidative stress and toxic compound production by immune cells [25].

182   Further, MSC produce ECM proteins, and factors of matrix turnover (matrix
183   metalloproteases etc.), as well as cytokines and growth factors to attract and support
184   other cell types (endothelial, tissue-specific progenitors etc.) [25-27].

185   This guided to investigate the composition of MSC secretome and our group
186   as well as others found that physiological potency of secretome is sufficient to
187   trigger events crucial for regeneration [28-30]. It activates proliferation and survival
188   of different cells under stress, protects tissue from excessive fibrosis, induces
189   angiogenesis and nerve growth, regulates immune response etc. leading to idea of
190   secretome therapeutic application known as "cell-free cell therapy" [31-33]. Our
191   further efforts were focused on functionalization of MSC secretome by viral delivery
192   of growth factors genes or pre-treatment by recombinant proteins. And we have
193   shown that it results in significant shift of MSC functional activity and improvement
194   of therapeutic effect [34-36]. However, we did not find increase of engraftment after
195   functionalization leading us to a generally supported conclusion that MSC therapies
196   succeed to deliver secretome produced by stem cells over time rather than replenish
197   native tissue elements by their differentiation.

198

199   **Table 1. Biological properties of MSC that contribute to their potential**
200   **as participants of feeder formation**

MSC property/function	Reference
Ubiquitous location in tissues and organs	Bianco, et al. [37], Caplan, et al. [38]
Multipotency and high proliferative potential	Pittenger, et al. [39] Kalinina, et al. [40]
Survival under stress conditions and inflammation	Lee, et al. [41] Oh, et al. [42] Grigoryeva, et al.
Immunomodulation and reduction of inflammation	Bernardo and Fibbe [26] Spaeth, et al. [43] Semedo, et al. [44]

Active pleiotropic secretome comprising of ECM components, growth factors, cytokines and extracellular vesicles	Kalinina, et al. [28] Konala, et al. [29] Vizoso, et al. [32]
Ability to support and regulate other cell types *in vivo*	Rubina, et al. [27] Battiwalla, et al. [45]

Thus, after injury damage-activated MSC mitigate inflammatory response and produce basic elements of the putative *feeder*: ECM and soluble trophic factors to attract other cells, to interact with them, to form a basis for regeneration.

Nevertheless, one may wonder why MSC delivered by injection fail to form interactions between each other and drive regeneration. Available answer is that negative influence of ECM and contacts disruption due to harvesting from monolayer followed by injection makes MSC unable to perform their functions [46]. It is predominantly related to stress due to loss of microenvironment that existed *in vitro*, namely − adhesive surface, ECM and soluble factors in culture medium. Certain reports claim that after injection up to 70% of MSC undergo cell death within the next 48-72 hours after delivery [40].

Thus, MSC therapy is limited to bystander effects similar to other stem cell therapies. However, MSC are able to produce basic components of their own microenvironment: ECM and pro-survival soluble factors. Moreover, they are able to organize this microenvironment and generate a tissue-like structure *in vitro* – namely, cell sheet (CS). The latter is generally used to facilitate cell delivery and transplant MSC in an organized manner resolving the problem of dispersed cells' inability to survive without appropriate microenvironment. Below we shall provide our point of view and data that support CS-based MSC delivery as a more physiological approach potentially providing ground for effective human regeneration.

4. Cell sheet technology: basics and application

Cell sheets are scaffold-free tissue-like constructs consisting of living cells, ECM and soluble factors accumulated in routine culture conditions. Formation of

227 CS varies in time (3-14 days), depends on ECM and soluble factors production, cells

228 proliferation and their ability to integrate to a multilayered construct that detaches

229 from thermo-responsive coating [47] or after treatment by chelating agents and/or

230 mechanical peeling [48].

231   This technology quickly found application in many areas of regenerative

232 medicine, primarily due to advantages over direct injection. Delivery by CS is

233 positioned as a method to deliver cells along with microenvironment formed *in vitro*.

234 Many preclinical and clinical studies report CS efficacy to treat diseases of the

235 bones, cartilage [49, 50], skin, urinary bladder, heart, blood vessels, esophagus,

236 cornea etc. [51]. To better understand details and therapeutic promise of CS

237 technology in regenerative medicine we may address the Reader to a number of

238 recent seminal reviews [52-56].

239   Experimental studies to treat wounds, limb ischemia [57], nerve damage [58]

240 and myocardial infarction [59] have been published by our group during last decade.

241 Besides delivery of primary MSC sheets we have studied endothelial cells, cardiac

242 stem cells [48] and viral modification of CS to enhance therapeutic outcomes [57,

243 58, 60] and our data along with published works of other researchers allows two

244 general conclusions regarding CS as a delivery platform for MSC therapy.

245   *1. Delivery of MSC assembled as CS results in strong and reproducible*

246 *increase of efficacy in models of different tissue lesions.*

247   Comparison of efficacy after delivery of equivalent amount of MSC by

248 injection or by CS transplantation has shown that sheets are superior to injected cells.

249 In our studies CS have performed better than suspended MSC for treatment of

250 ischemia, nerve repair [57, 58, 61], deep wound healing [62] and pressure ulcer (data

251 unpublished). Recent papers describing use of CS for liver regeneration [63] or bone

252 healing [64] often omit direct "head to head" comparison of outcomes after CS or

253 suspended delivery yet most of them report long-term survival of transplanted cells

254 suggesting that it mediates therapeutic outcome.

255   *2. MSC delivered as CS show signs of engraftment including long-term*

256 *survival (over 14 days), vascularization of CS and proliferation of delivered cells.*

257 This point was illustrated in one of our works using subcutaneous
258 implantation of labeled MSC sheets in mice indicating CD31-positive capillaries and
259 larger caliber blood vessels in CS mass as well as Ki-67-positive MSC detected 2
260 weeks after transplantation [57]. Our results were supported by data from other
261 groups using MSC sheets [65-67] and by own data using other cell types – namely
262 cardiac stem cell sheets transplanted to treat myocardial infarction in a rat model
263 [48, 59].

264 Points mentioned above support our suggestion that assembly of CS from
265 MSC facilitates *engraftment* and, thus, delivery of a stromal part of suggested feeder
266 that may become a putative basis for further regeneration. However, to do this MSC
267 must interact with other cell types and integrate in an organotypic structure.
268 Potential mechanism for that is generation of stimuli gradients which will be
269 explained further.

270

5. Role of MSC in structure organization in development and regeneration

273 Early stages of human development are driven by embryonic stem cells'
274 (ESCs) ability to interconnect and integrate in community [68]. Crosstalk between
275 ESCs dynamically changes form and structure resulting in embryogenesis.
276 Mesenchyme appears during gastrulation phase and gets involved in subsequent
277 organogenesis. Interactions of mesenchymal cells with epithelial cells are crucial in
278 development of teeth [69], lungs [70], pancreas [71], heart [72], kidney [73, 74] and
279 liver [75].

280 Critical aspect of mentioned processes in development is formation of stimuli
281 gradients including those produced by mesenchymal cells [76]. Soluble factors,
282 ECM and cell-to-cell contacts have been postulated to participate in accurate
283 organogenesis of many organs and role of mesenchymal cells acting as main
284 structure organizers is well-established as well [77, 78].

285 In postnatal period MSC (despite losing many traits they featured in
286 development) may retain organizing ability they use for regeneration and molecular

287   basis of this ability is generation of stimuli gradients once regenerative program is

288   activated by injury. This supports our concept of MSC role in generation of putative

289   feeder and explain how they can facilitate and "map" next stages of regeneration.

290

6. MSC as organizers of other cell types: *ex vivo* evidence

292   MSC's ability to support and organize other cells *in vitro* has been reported

293   previously for epithelial cells from embryonic salivary gland. In this study MSC but

294   not conventionally used feeder (NIH/3T3 fibroblasts) supported self-assembly of

295   primary embryonic epithelial cells and subsequent branching with buds formation

296   in 3D culture resembling mesenchyme function in development [79].

297   One of the strongest evidences of MSC ability to facilitate organogenesis *in

298   vitro* is a work by Takebe et al. [80] describing MSC-driven condensation of iPS-

299   derived progenitors to form bud-shaped organoids. Spectrum of organoids that can

300   be successfully assembled under influence of MSC *in vitro* included liver, intestine,

301   lung, kidney, heart and brain (!). Finally, endothelium-containing pancreatic and

302   renal buds derived under influence of MSC were transplanted to animals where they

303   rapidly underwent vascularization and self-organized into functional, tissue-specific

304   structures.

305   Organization of such complex organ(oid)s as pancreas or kidney should

306   employs the same principles as in organogenesis relying on focal stimuli provided

307   by MSC to organize other cell types in a proper manner. At this point our recent data

308   describing heterogeneity in human MSC-derived CS provides a new outlook on this

309   delivery mode and its potential impact on regeneration [81, 82].

310   In our study a CS from human adipose-derived MSC showed significant

311   morphological heterogeneity with 2 distinct compartments of high and low density

312   of cells ("hills" and "valleys respectively – see Figure 2, a-c). High-density regions

313   were formed as a result of MSC proliferation cycles followed by coordinated

314   contraction and migration of cells (see Supplementary video 1) which resembles a

315   previously described contraction phenomenon that accompanies MSC-driven self-
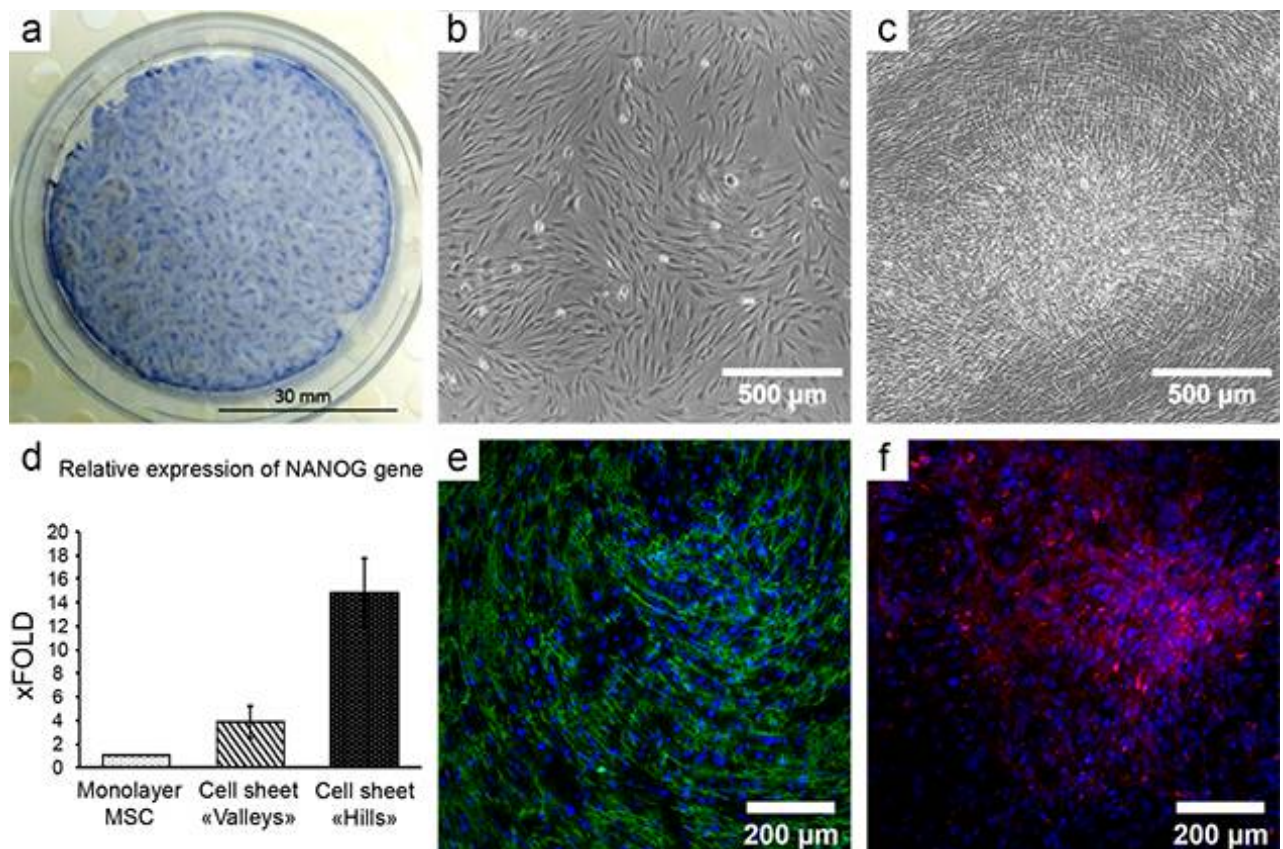
316   organization.

Figure 2. Self-organization of MSC in cell sheets results in formation of heterogeneous microenvironment.

a. Heterogeneous distribution of MSC in CS at day 12 of cultures; hematoxylin staining;

b-c. Microphotographs taken in the same field of view of MSC culture at day 1 (b) and day 12 (c). Formation of a high-density "hill" is observed as a result of MSC self-organization in CS; time-lapse microscopy, phase-contrast.

d. MSC subpopulations were picked by laser microdissection. MSC in the "hills" show increased expression of stemness-associated factor (NANOG); real-time qPCR (n=3); data expressed as mean ± standard deviation are shown.

e-f. MSC in CS organise EDA-fibronectin in regular network of fibres (green, panel e) while laminin (red, panel f) is deposited primarily in "hills", but not in "valleys" creating foci of ECM deposition in CS (f); immunofluorescent microscopy, nuclei are stained by DAPI (blue); from Nimiritsky et al. [81]. For details of experimental procedures please address supplementary Materials and methods online.

335 Mesenchyme-mediated guidance in development relies on ECM differential
336 distribution that has crucial importance as it facilitates attraction and retain of
337 different cell types [77]. We found that in MSC sheets two ECM proteins – EDA-
338 fibronectin and laminin are distributed uneven with higher density in "hills" and
339 lower – in "valleys" (Fig. 2, e and f). Condensed laminin deposition was detectable
340 only in "hills" of CS and in monolayer of MSC no acquirable signal was detected
341 (data not shown). Laminin is known to mediate interaction of mesenchymal and
342 epithelial cells leading to assumption that heterogeneity of its in CS reflects
343 formation of guiding ECM stimuli that can orchestrate organization of endothelial,
344 epithelial and mature stromal cells on this *in vitro* formed feeder.

345 Differential morphology and ECM deposition in CS suggest that different
346 MSC subtypes main retained in them so we used laser microdissection to separate
347 "hills" and "valleys" at day 12 to evaluate relative expression of stemness-related
348 gene *NANOG* in MSC from different microenvironments.

349 We compared laser-dissected "hills" vs. remaining "valley" portion of CS and
350 found significantly (up to 15-fold) increase of *NANOG* expression compared to
351 "valleys" of same CS (Figure 2, d). It suggested that heterogeneity of CS results in
352 either sorting of different cells to different compartments of the construct or local
353 shift of stemness *in situ* resembling "mapping" function of mesenchyme known in
354 development and suggesting that MSC sheets actually can be considered as a guiding
355 construct rather than just delivery tool.

356 Previously MSC have been successfully used to generate pre-vascularized
357 structures and endothelial cells spontaneously organized in tubes once were co-
358 seeded with MSC for sheet formation [79, 83]. This provided a method for
359 fabrication of tissue-like 3D structures by stacking pre-vascularized CS together
360 without loss of nutrition inside the construct. Nevertheless, our data suggests more
361 attention to potential use of MSC-based CS as an effective driver of organoid or
362 tissue self-assembly as it possesses an important "mapping" trait required for
363 organization of other cell types by gradients of stimuli (Fig. 3).

364   Previous data obtained by our group and other authors using single cell
365 analysis found numerous subpopulations in primary MSC with varying sensitivity
366 to hormones, differentiation ability and signaling [84-86]. However, origin of
367 stimuli gradients and CS heterogeneity is subject for elucidation taking into account
368 data on mesenchymal cells' role in differentiation control during development and
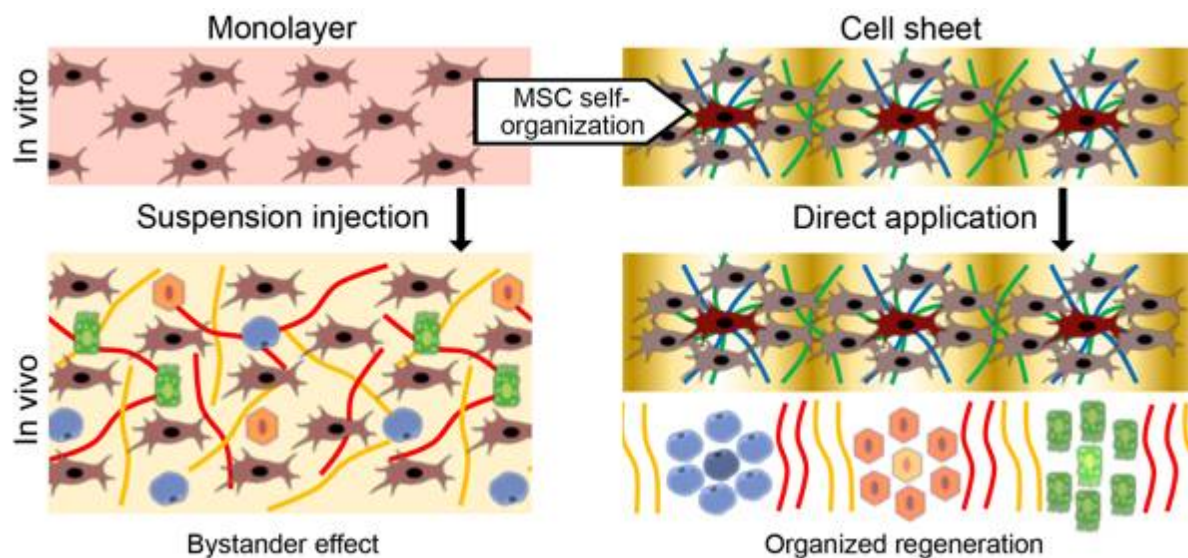369 organogenesis *ex vivo*.

370
371   **Figure 3. Self-organization of MSC as a basis of its organizing function**
372 **in regeneration.**

373   Conventionally used injection of MSC cultured in monolayer (left part) is
374 limited to paracrine (bystander) effects of delivered cells. Self-organization during
375 CS formation results in retain of microenvironment with gradient of stimuli that
376 organize regeneration after delivery (right part).

377

378   **7. Concluding remarks**

379   Present paper reflects our vision on how stem cell delivery might be improved
380 basing on knowledge of human development, system biology and – in particular –
381 limitations of existing cell therapy approaches.

382   To our knowledge regeneration after injury has not been previously compared
383 to the recovery of ecosystem structure. Terminology of ecological and evolutionary
384 biology has been extensively used in cancer [87-89] and stem cell [90] biology, so

385 we consider our approach reasonable to explain importance of putative feeder
386 formed in early stages of regeneration for its outcome.

387   In our view participation and recruitment of MSC during formation of feeder
388 has physiological rationale. Our point is supported by "mapping" role of
389 mesenchymal cells in development and by prominent examples suggesting that adult
390 MSC retain this ability even *ex vivo* driving functional organoid formations and
391 vascularization in a dish. The concept of feeder as a base layer for successful
392 regeneration might be a subject for discussion yet we suggest it as a summary of our
393 knowledge about regeneration and data from other groups.

394   This concept overall shifts our understanding of MSC-based CS as a delivery
395 platform for regenerative medicine. We believe that CS is not only a mean of "cells
396 + ECM" deployment, but may present a unique object for study of mammalian
397 regeneration, organoid assembly or tissue modelling. MSC within CS may retain or
398 reproduce certain traits of mesenchymal cells in development to orchestrate
399 regeneration. Origins and consequences of heterogeneity we observed in CS as well
400 as its influence on other cells (including tissue-specific progenitors and terminally
401 differentiated cells) is a subject for further investigation.

402

403   **Funding statement**

408

409 **References**

410 1.   Rao, M.; Mason, C.; Solomon, S., Cell therapy worldwide: an incipient
411   revolution. *Regen Med* **2015,** 10, (2), 181-91.
412 2.   Macrin, D.; Joseph, J. P.; Pillai, A. A.; Devi, A., Eminent Sources of Adult
413   Mesenchymal Stem Cells and Their Therapeutic Imminence. *Stem Cell Rev*
414   **2017,** 13, (6), 741-756.
415 3.   King, N. M.; Perrin, J., Ethical issues in stem cell research and therapy. *Stem*
416   *Cell Res Ther* **2014,** 5, (4), 85.

417 4.   Volarevic, V.; Markovic, B. S.; Gazdic, M.; Volarevic, A.; Jovicic, N.;
418   Arsenijevic, N.; Armstrong, L.; Djonov, V.; Lako, M.; Stojkovic, M.,
419   Ethical and Safety Issues of Stem Cell-Based Therapy. *Int J Med Sci* **2018,**
420   15, (1), 36-45.
421 5.   Schroeder, I. S., Stem cells: are we ready for therapy? *Methods Mol Biol*
422   **2014,** 1213, 3-21.
423 6.   Baksh, D.; Song, L.; Tuan, R. S., Adult mesenchymal stem cells:
424   characterization, differentiation, and application in cell and gene therapy. *J*
425   *Cell Mol Med* **2004,** 8, (3), 301-16.
426 7.   Gharaibeh, B.; Lavasani, M.; Cummins, J. H.; Huard, J., Terminal
427   differentiation is not a major determinant for the success of stem cell therapy
428   - cross-talk between muscle-derived stem cells and host cells. *Stem Cell Res*
429   *Ther* **2011,** 2, (4), 31.
430 8.   Savukinas, U. B.; Enes, S. R.; Sjoland, A. A.; Westergren-Thorsson, G.,
431   Concise Review: The Bystander Effect: Mesenchymal Stem Cell-Mediated
432   Lung Repair. *Stem Cells* **2016,** 34, (6), 1437-44.
433 9.   Breunig, J. J.; Arellano, J. I.; Macklis, J. D.; Rakic, P., Everything that
434   glitters isn't gold: a critical review of postnatal neural precursor analyses.
435   *Cell Stem Cell* **2007,** 1, (6), 612-27.
436 10. Morrison, S. J.; Spradling, A. C., Stem cells and niches: mechanisms that
437   promote stem cell maintenance throughout life. *Cell* **2008,** 132, (4), 598-
438   611.
439 11. Schofield, R., The relationship between the spleen colony-forming cell and
440   the haemopoietic stem cell. *Blood Cells* **1978,** 4, (1-2), 7-25.
441 12. Scadden, D. T., The stem-cell niche as an entity of action. *Nature* **2006,** 441,
442   (7097), 1075-9.
443 13. Tanaka, E. M.; Reddien, P. W., The cellular basis for animal regeneration.
444   *Dev Cell* **2011,** 21, (1), 172-85.
445 14. Kurtz, A., Mesenchymal stem cell delivery routes and fate. *Int J Stem Cells*
446   **2008,** 1, (1), 1-7.
447 15. Eggenhofer, E.; Luk, F.; Dahlke, M. H.; Hoogduijn, M. J., The life and fate
448   of mesenchymal stem cells. *Front Immunol* **2014,** 5, 148.
449 16. Young, J. A.; Evans, R. A., Population Dynamics after Wildfires in
450   Sagebrush Grasslands. *Journal of Range Management* **1978,** 31, (4), 283-
451   289.
452 17. Sutherland, E. F.; Dickman, C. R., Mechanisms of recovery after fire by
453   rodents in the Australian environment: a review. *Wildlife Research* **1999,** 26,
454   (4), 405-419.
455 18. McCusker, C. D.; Gardiner, D. M., Positional Information Is Reprogrammed
456   in Blastema Cells of the Regenerating Limb of the Axolotl (Ambystoma
457   mexicanum). *PLoS ONE* **2013,** 8, (9).
458 19. Thomas, E. D.; Lochte, H. L., Jr.; Lu, W. C.; Ferrebee, J. W., Intravenous
459   infusion of bone marrow in patients receiving radiation and chemotherapy.
460   *N Engl J Med* **1957,** 257, (11), 491-6.

461   20.   Loi, R.; Beckett, T.; Goncz, K. K.; Suratt, B. T.; Weiss, D. J., Limited
462   restoration of cystic fibrosis lung epithelium in vivo with adult bone
463   marrow-derived cells. *Am J Respir Crit Care Med* **2006,** 173, (2), 171-9.
464   21.   Klimczak, A.; Kozlowska, U., Mesenchymal stromal cells and tissue-
465   specific progenitor cells: their role in tissue homeostasis. *Stem cells*
466   *international* **2016,** 2016.
467   22.   Kosinski, C.; Li, V. S.; Chan, A. S.; Zhang, J.; Ho, C.; Tsui, W. Y.; Chan, T.
468   L.; Mifflin, R. C.; Powell, D. W.; Yuen, S. T., Gene expression patterns of
469   human colon tops and basal crypts and BMP antagonists as intestinal stem
470   cell niche factors. *Proceedings of the National Academy of Sciences* **2007,**
471   104, (39), 15418-15423.
472   23.   Kfoury, Y.; Scadden, D. T., Mesenchymal cell contributions to the stem cell
473   niche. *Cell stem cell* **2015,** 16, (3), 239-253.
474   24.   Caplan, A. I., Mesenchymal Stem Cells: Time to Change the Name! *Stem*
475   *Cells Transl Med* **2017,** 6, (6), 1445-1451.
476   25.   Dimarino, A. M.; Caplan, A. I.; Bonfield, T. L., Mesenchymal stem cells in
477   tissue repair. *Front Immunol* **2013,** 4, 201.
478   26.   Bernardo, M. E.; Fibbe, W. E., Mesenchymal stromal cells: sensors and
479   switchers of inflammation. *Cell stem cell* **2013,** 13, (4), 392-402.
480   27.   Rubina, K.; Kalinina, N.; Efimenko, A.; Lopatina, T.; Melikhova, V.;
481   Tsokolaeva, Z.; Sysoeva, V.; Tkachuk, V.; Parfyonova, Y., Adipose stromal
482   cells stimulate angiogenesis via promoting progenitor cell differentiation,
483   secretion of angiogenic factors, and enhancing vessel maturation. *Tissue*
484   *Engineering Part A* **2009,** 15, (8), 2039-2050.
485   28.   Kalinina, N.; Kharlampieva, D.; Loguinova, M.; Butenko, I.; Pobeguts, O.;
486   Efimenko, A.; Ageeva, L.; Sharonov, G.; Ischenko, D.; Alekseev, D.;
487   Grigorieva, O.; Sysoeva, V.; Rubina, K.; Lazarev, V.; Govorun, V.,
488   Characterization of secretomes provides evidence for adipose-derived
489   mesenchymal stromal cells subtypes. *Stem Cell Res Ther* **2015,** 6, 221.
490   29.   Konala, V. B.; Mamidi, M. K.; Bhonde, R.; Das, A. K.; Pochampally, R.;
491   Pal, R., The current landscape of the mesenchymal stromal cell secretome:
492   A new paradigm for cell-free regeneration. *Cytotherapy* **2016,** 18, (1), 13-
493   24.
494   30.   Lopatina, T.; Bruno, S.; Tetta, C.; Kalinina, N.; Porta, M.; Camussi, G.,
495   Platelet-derived growth factor regulates the secretion of extracellular
496   vesicles by adipose mesenchymal stem cells and enhances their angiogenic
497   potential. *Cell Commun Signal* **2014,** 12, 26.
498   31.   Sagaradze, G. D.; Basalova, N. A.; Kirpatovsky, V. I.; Ohobotov, D. A.;
499   Grigorieva, O. A.; Balabanyan, V. Y.; Kamalov, A. A.; Efimenko, A. Y.,
500   Application of rat cryptorchidism model for the evaluation of mesenchymal
501   stromal cell secretome regenerative potential. *Biomed Pharmacother* **2019,**
502   109, 1428-1436.
503   32.   Vizoso, F. J.; Eiro, N.; Cid, S.; Schneider, J.; Perez-Fernandez, R.,
504   Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic
505   Strategies in Regenerative Medicine. *Int J Mol Sci* **2017,** 18, (9).

33. Sagaradze, G. D.; Nimiritsky, P. P.; Akopyan, Z. A.; Makarevich, P. I.; Efimenko, A. Y., "Cell-Free Therapeutics" from Components Secreted by Mesenchymal Stromal Cells as a Novel Class of Biopharmaceuticals. In *Biopharmaceuticals*, Chen, M.-K. Y. a. Y.-C., Ed. InTechOpen: 2018.

34. Zubkova, E. S.; Beloglazova, I. B.; Makarevich, P. I.; Boldyreva, M. A.; Sukhareva, O. Y.; Shestakova, M. V.; Dergilev, K. V.; Parfyonova, Y. V.; Menshikov, M. Y., Regulation of Adipose Tissue Stem Cells Angiogenic Potential by Tumor Necrosis Factor-Alpha. *J Cell Biochem* **2016,** 117, (1), 180-96.

35. Shevchenko, E. K.; Makarevich, P. I.; Tsokolaeva, Z. I.; Boldyreva, M. A.; Sysoeva, V. Y.; Tkachuk, V. A.; Parfyonova, Y. V., Transplantation of modified human adipose derived stromal cells expressing VEGF165 results in more efficient angiogenic response in ischemic skeletal muscle. *J Transl Med* **2013,** 11, 138.

36. Zubkova, E. S.; Semenkova, L. N.; Dudich, I. V.; Dudich, E. I.; Khromykh, L. M.; Makarevich, P. I.; Parfenova, E. V.; Men'shikov, M., [Recombinant human alpha-fetoprotein as a regulator of adipose tissue stromal cell activity]. *Bioorg Khim* **2012,** 38, (5), 524-34.

37. Bianco, P.; Cao, X.; Frenette, P. S.; Mao, J. J.; Robey, P. G.; Simmons, P. J.; Wang, C.-Y., The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. *Nature medicine* **2013,** 19, (1), 35.

38. Caplan, A. I., Mesenchymal Stem Cells: The Past, the Present, the Future. *Cartilage* **2010,** 1, (1), 6-9.

39. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; Moorman, M. A.; Simonetti, D. W.; Craig, S.; Marshak, D. R., Multilineage potential of adult human mesenchymal stem cells. *science* **1999,** 284, (5411), 143-147.

40. Kalinina, N. I.; Sysoeva, V. Y.; Rubina, K. A.; Parfenova, Y. V.; Tkachuk, V. A., Mesenchymal stem cells in tissue growth and repair. *Acta Naturae* **2011,** 3, (4), 30-7.

41. Lee, S.; Choi, E.; Cha, M.-J.; Hwang, K.-C., Cell adhesion and long-term survival of transplanted mesenchymal stem cells: a prerequisite for cell therapy. *Oxidative medicine and cellular longevity* **2015,** 2015.

42. Oh, E. J.; Lee, H. W.; Kalimuthu, S.; Kim, T. J.; Kim, H. M.; Baek, S. H.; Zhu, L.; Oh, J. M.; Son, S. H.; Chung, H. Y., In vivo migration of mesenchymal stem cells to burn injury sites and their therapeutic effects in a living mouse model. *Journal of Controlled Release* **2018,** 279, 79-88.

43. Spaeth, E.; Klopp, A.; Dembinski, J.; Andreeff, M.; Marini, F., Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene therapy* **2008,** 15, (10), 730.

44. Semedo, P.; Palasio, C. G.; Oliveira, C. D.; Feitoza, C. Q.; Gonçalves, G. M.; Cenedeze, M. A.; Wang, P. M.; Teixeira, V. P.; Reis, M. A.; Pacheco-Silva, A., Early modulation of inflammation by mesenchymal stem cell after

acute kidney injury. *International immunopharmacology* **2009,** 9, (6), 677-682.

45. Battiwalla, M.; Hematti, P., Mesenchymal stem cells in hematopoietic stem cell transplantation. *Cytotherapy* **2009,** 11, (5), 503-15.

46. Kushida, A.; Yamato, M.; Isoi, Y.; Kikuchi, A.; Okano, T., A noninvasive transfer system for polarized renal tubule epithelial cell sheets using temperature-responsive culture dishes. *Eur Cell Mater* **2005,** 10, 23-30; discussion 23-30.

47. Okano, T.; Yamada, N.; Sakai, H.; Sakurai, Y., A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). *J Biomed Mater Res* **1993,** 27, (10), 1243-51.

48. Dergilev, K. V.; Makarevich, P. I.; Tsokolaeva, Z. I.; Boldyreva, M. A.; Beloglazova, I. B.; Zubkova, E. S.; Menshikov, M. Y.; Parfyonova, Y. V., Comparison of cardiac stem cell sheets detached by Versene solution and from thermoresponsive dishes reveals similar properties of constructs. *Tissue Cell* **2017,** 49, (1), 64-71.

49. Chen, L.; Xing, Q.; Zhai, Q.; Tahtinen, M.; Zhou, F.; Chen, L.; Xu, Y.; Qi, S.; Zhao, F., Pre-vascularization Enhances Therapeutic Effects of Human Mesenchymal Stem Cell Sheets in Full Thickness Skin Wound Repair. *Theranostics* **2017,** 7, (1), 117-131.

50. Roh, J. L.; Lee, J.; Kim, E. H.; Shin, D., Plasticity of oral mucosal cell sheets for accelerated and scarless skin wound healing. *Oral Oncol* **2017,** 75, 81-88.

51. Kirby, G. T. S.; Michelmore, A.; Smith, L. E.; Whittle, J. D.; Short, R. D., Cell sheets in cell therapies. *Cytotherapy* **2018,** 20, (2), 169-180.

52. Li, M.; Ma, J.; Gao, Y.; Yang, L., Cell sheet technology: a promising strategy in regenerative medicine. *Cytotherapy* **2018**.

53. Masuda, S.; Shimizu, T., Three-dimensional cardiac tissue fabrication based on cell sheet technology. *Adv Drug Deliv Rev* **2016,** 96, 103-9.

54. Hannachi, I. E.; Yamato, M.; Okano, T., Cell sheet technology and cell patterning for biofabrication. *Biofabrication* **2009,** 1, (2), 022002.

55. Matsuura, K.; Utoh, R.; Nagase, K.; Okano, T., Cell sheet approach for tissue engineering and regenerative medicine. *J Control Release* **2014,** 190, 228-39.

56. Dergilev, K. V.; Makarevich, P. I.; Menshikov, M. Y.; Parfyonova, E. V., Application of tissue engineered constructs on the basis of cell sheets for restoration of tissues and organs. *Genes and Cells* **2016,** 11, (3), 23-32.

57. Makarevich, P. I.; Boldyreva, M. A.; Gluhanyuk, E. V.; Efimenko, A. Y.; Dergilev, K. V.; Shevchenko, E. K.; Sharonov, G. V.; Gallinger, J. O.; Rodina, P. A.; Sarkisyan, S. S., Enhanced angiogenesis in ischemic skeletal muscle after transplantation of cell sheets from baculovirus-transduced adipose-derived stromal cells expressing VEGF165. *Stem cell research & therapy* **2015,** 6, (1), 204.

58. Hsu, M. N.; Liao, H. T.; Li, K. C.; Chen, H. H.; Yen, T. C.; Makarevich, P.; Parfyonova, Y.; Hu, Y. C., Adipose-derived stem cell sheets functionalized

595   by hybrid baculovirus for prolonged GDNF expression and improved nerve
596   regeneration. *Biomaterials* **2017,** 140, 189-200.

597 59. Dergilev, K.; Tsokolaeva, Z.; Makarevich, P.; Beloglazova, I.; Zubkova, E.;
598   Boldyreva, M.; Ratner, E.; Dyikanov, D.; Menshikov, M.; Ovchinnikov, A.;
599   Ageev, F.; Parfyonova, Y., C-Kit Cardiac Progenitor Cell Based Cell Sheet
600   Improves Vascularization and Attenuates Cardiac Remodeling following
601   Myocardial Infarction in Rats. *Biomed Res Int* **2018,** 2018, 3536854.

602 60. Yeh, T. S.; Fang, Y. H.; Lu, C. H.; Chiu, S. C.; Yeh, C. L.; Yen, T. C.;
603   Parfyonova, Y.; Hu, Y. C., Baculovirus-transduced, VEGF-expressing
604   adipose-derived stem cell sheet for the treatment of myocardium infarction.
605   *Biomaterials* **2014,** 35, (1), 174-84.

606 61. Makarevich, P. I.; Boldyreva, M. A.; Dergilev, K. V.; Gluhanyuk, E. V.;
607   Gallinger, J. O.; Efimenko, A. Y.; Tkachuk, V. A.; Parfyonova, Y. V.,
608   Transplantation of cell sheets from adipose-derived mesenchymal stromal
609   cells effectively induces angiogenesis in ischemic skeletal muscle. *Genes*
610   *and Cells* **2015,** 10, (3), 68-77.

611 62. Alexandrushkina, N. A.; Makarevich, O. A.; Nimiritskiy, P. P.; Efimenko,
612   A. Y.; Danilova, N. V.; Parfyonova, Y. V.; Makarevich, P. I.; Tkachuk, V.
613   A., Adipose mesenchymal stromal cell sheets accelerate healing in rat model
614   of deep wound. *Hum Gene Ther* **2017,** 28, (12), A86-A86.

615 63. Tatsumi, K.; Okano, T., Hepatocyte Transplantation: Cell Sheet Technology
616   for Liver Cell Transplantation. *Curr Transplant Rep* **2017,** 4, (3), 184-192.

617 64. Zhou, Y.; Chen, F.; Ho, S. T.; Woodruff, M. A.; Lim, T. M.; Hutmacher, D.
618   W., Combined marrow stromal cell-sheet techniques and high-strength
619   biodegradable composite scaffolds for engineered functional bone grafts.
620   *Biomaterials* **2007,** 28, (5), 814-24.

621 65. Chen, G.; Qi, Y.; Niu, L.; Di, T.; Zhong, J.; Fang, T.; Yan, W., Application
622   of the cell sheet technique in tissue engineering. *Biomed Rep* **2015,** 3, (6),
623   749-757.

624 66. Li, L.; Chen, X.; Wang, W. E.; Zeng, C., How to Improve the Survival of
625   Transplanted Mesenchymal Stem Cell in Ischemic Heart? *Stem Cells Int*
626   **2016,** 2016, 9682757.

627 67. Tano, N.; Narita, T.; Kaneko, M.; Ikebe, C.; Coppen, S. R.; Campbell, N.
628   G.; Shiraishi, M.; Shintani, Y.; Suzuki, K., Epicardial placement of
629   mesenchymal stromal cell-sheets for the treatment of ischemic
630   cardiomyopathy; in vivo proof-of-concept study. *Mol Ther* **2014,** 22, (10),
631   1864-71.

632 68. Li, N.; Xie, T.; Sun, Y., Towards organogenesis and morphogenesis in vitro:
633   harnessing engineered microenvironment and autonomous behaviors of
634   pluripotent stem cells. *Integrative Biology* **2018,** 10, (10), 574-586.

635 69. Grobstein, C., Epithelio-mesenchymal specificity in the morphogenesis of
636   mouse sub-mandibular rudiments in vitro. *Journal of Experimental Zoology*
637   **1953,** 124, (2), 383-413.

638 70. Herriges, M.; Morrisey, E. E., Lung development: orchestrating the
639   generation and regeneration of a complex organ. *Development* **2014,** 141,
640   (3), 502-13.
641 71. Golosow, N.; Grobstein, C., Epitheliomesenchymal interaction in pancreatic
642   morphogenesis. *Developmental biology* **1962,** 4, (2), 242-255.
643 72. Wessels, A.; Anderson, R. H.; Markwald, R. R.; Webb, S.; Brown, N. A.;
644   Viragh, S.; Moorman, A. F.; Lamers, W. H., Atrial development in the
645   human heart: an immunohistochemical study with emphasis on the role of
646   mesenchymal tissues. *Anat Rec* **2000,** 259, (3), 288-300.
647 73. Hatini, V.; Huh, S. O.; Herzlinger, D.; Soares, V. C.; Lai, E., Essential role
648   of stromal mesenchyme in kidney morphogenesis revealed by targeted
649   disruption of Winged Helix transcription factor BF-2. *Genes & development*
650   **1996,** 10, (12), 1467-1478.
651 74. Hum, S.; Rymer, C.; Schaefer, C.; Bushnell, D.; Sims-Lucas, S., Ablation of
652   the renal stroma defines its critical role in nephron progenitor and
653   vasculature patterning. *PloS one* **2014,** 9, (2), e88400.
654 75. Yin, C.; Evason, K. J.; Asahina, K.; Stainier, D. Y., Hepatic stellate cells in
655   liver development, regeneration, and cancer. *J Clin Invest* **2013,** 123, (5),
656   1902-10.
657 76. Gilbert, S. F., *Developmental Biology*. Sinauer: 2016.
658 77. C Birchmeier, a.; Birchmeier, W., Molecular Aspects of Mesenchymal-
659   Epithelial Interactions. *Annual Review of Cell Biology* **1993,** 9, (1), 511-540.
660 78. Green, J. B.; Smith, J. C., Growth factors as morphogens: do gradients and
661   thresholds establish body plan? *Trends in genetics : TIG* **1991,** 7, (8), 245-
662   50.
663 79. Ren, L.; Ma, D.; Liu, B.; Li, J.; Chen, J.; Yang, D.; Gao, P., Preparation of
664   three-dimensional vascularized MSC cell sheet constructs for tissue
665   regeneration. *Biomed Res Int* **2014,** 2014, 301279.
666 80. Takebe, T.; Enomura, M.; Yoshizawa, E.; Kimura, M.; Koike, H.; Ueno, Y.;
667   Matsuzaki, T.; Yamazaki, T.; Toyohara, T.; Osafune, K.; Nakauchi, H.;
668   Yoshikawa, H. Y.; Taniguchi, H., Vascularized and Complex Organ Buds
669   from Diverse Tissues via Mesenchymal Cell-Driven Condensation. *Cell*
670   *Stem Cell* **2015,** 16, (5), 556-65.
671 81. Nimiritsky, P. P.; Alexandrushkina, N. A.; Sagaradze, G. D.; Tyurin-
672   Kuzmin, P. A.; Efimenko, A. Y.; Makarevich, P. I., MSC self-organization
673   in vitro is concordant with elevation of regenerative potential and
674   characteristics related to stem cell niche function. *Hum Gene Ther* **2018,** 29,
675   (12), A62-A62.
676 82. Nimiritsky, P. P.; Makarevich, O. A.; Sagaradze, G. D.; Efimenko, A. Y.;
677   Eremichev, R. Y.; Makarevich, P. I.; Tkachuk, V. A., Evaluation of
678   mechanisms underlying regenerative potential of cell sheets from human
679   mesenchymal stromal cells. *Hum Gene Ther* **2017,** 28, (12), A100-A100.
680 83. Zhou, Y.; Dong, L. Q.; Liu, C.; Lin, Y. H.; Yu, M. F.; Ma, L.; Zhang, B.;
681   Cheng, K.; Weng, W. J.; Wang, H. M., Engineering prevascularized

composite cell sheet by light-induced cell sheet technology. *Rsc Adv* **2017,** 7, (52), 32468-32477.

84. Bianco, P., "Mesenchymal" Stem Cells. *Annual Review of Cell and Developmental Biology* **2014,** 30, (1), 677-704.

85. Kotova, P. D.; Sysoeva, V. Y.; Rogachevskaja, O. A.; Bystrova, M. F.; Kolesnikova, A. S.; Tyurin-Kuzmin, P. A.; Fadeeva, J. I.; Tkachuk, V. A.; Kolesnikov, S. S., Functional expression of adrenoreceptors in mesenchymal stromal cells derived from the human adipose tissue. *Biochim Biophys Acta* **2014,** 1843, (9), 1899-908.

86. Tyurin-Kuzmin, P. A.; Fadeeva, J. I.; Kanareikina, M. A.; Kalinina, N. I.; Sysoeva, V. Y.; Dyikanov, D. T.; Stambolsky, D. V.; Tkachuk, V. A., Activation of beta-adrenergic receptors is required for elevated alpha1A-adrenoreceptors expression and signaling in mesenchymal stromal cells. *Sci Rep* **2016,** 6, 32835.

87. Gatenby, R. A., Population ecology issues in tumor growth. *Cancer Res* **1991,** 51, (10), 2542-7.

88. Nagy, J. D., The ecology and evolutionary biology of cancer: a review of mathematical models of necrosis and tumor cell diversity. *Math Biosci Eng* **2005,** 2, (2), 381-418.

89. Gatenby, R. A.; Grove, O.; Gillies, R. J., Quantitative imaging in cancer evolution and ecology. *Radiology* **2013,** 269, (1), 8-15.

90. Papayannopoulou, T.; Scadden, D. T., Stem-cell ecology and stem cells in motion. *Blood* **2008,** 111, (8), 3923-30.