Konvencionalna transplantacija matičnih ćelija hematopoeze (MĆH) je dobro poznat metod lečenja brojnih stečenih i urođenih poremećaja hematopoeze, poremećaja imunskog sistema, kao i određenih metaboličkih oboljenja. Matica ćelije (MC) se mogu definisati kao ćelije koje imaju sposobnost samoobnavljanja i koje poseduju visoki proliferativni kapacitet, kao i potencijal da se diferenciju u funkcionalno kompetentne zrele ćelije. MC se mogu podeliti na embrionalne MC (EMC) i tijekom diferenciranja, odnosno adultne MC – kao što su one iz kostne srži, plastične crve i krvi crve, kao i druge neematopoeet- ske odnose somatske ćelije. Kod odraslih, MC se obično smatraju ograničenim po pitanju njihovog regenerativnog potencijala i potencijala diferenciranja, dok su EMC „prave“ potencijalno potencijalne somatske ćelije, jer imaju sposobnost da se razviju u sva tri tipa embrionalnog tkiva u ljudskom organizmu. Među MC koje se regenerativno mogu presaditi, ove ćelije predstavljaju vrstu koja najviše obećava, ali i koja je najkontroverznija. Manje zrele ili primitivnije MĆH imaju potencijal da se diferenciraju, ne samo u sve vrste zrele ćelije krvi, već i u neke vrste somatskih ćelija (plastičnost MC). U razli- čitim kliničkim uslovima, transplantacija nezrelih (primitivnih) MC dovodi do repopulacije kostne srži autotransplantata, uz kasniju potpunu, stabilnu i dugoročnu rekonstruk- ciju hematopoze. Imajući u vidu da su primitivne ćelije takođe sposobne za ukamenjivanje (implantaciju) u različita tkiva, autologna transplantacija MC u oštećeno i/ili ishemično područje indukuje njihovo naseljavanje i slijedstveno transdiferenciranje u tkivna linije organa domaćina, uključujući i neovlašćivanje. Stoga su one klinički primjenljive u oblasti regenerativne medicine, u lečenju tkivnih oštećenja miokarda, mozga, krvnih sudova, jetre, pankreasa i drugih tkiva. Srna ovoj pregleda jeste rekompilacija ključnih otkrića u oblasti transplantacija MC, koja je u fazi ubrzanog razvoja. U radu je dat i pregled primene MC u tzv. konvencionalnim transplantacijama i u regenerativnoj medicini. Uz to, dat je i sažet kritički osvrt na naša sopstvena istraživanja u oblasti MĆ.

Ključne reči: matično tkivo, regenerativna medicine, autologna transplantacija, plasticnost matičnih ćelija, transplantacija, regenerativna medicine

ABSTRACT

Conventional hematopoietic stem cell transplantation is a well-known treatment method for numerous acquired and congenital hematopoietic disorders, disorders of the immune system, as well as certain metabolic disorders. Stem cells (SCs) can be defined as cells capable of self-renewal with a high proliferative capacity and the potential to differentiate into functionally competent mature cells. Stem cells can be divided into embryonic SCs (ESCs) and tissue-specific or adult SCs – such as bone marrow (BM) stem cells, peripheral blood (PB) stem cells, and SCs derived from umbilical cord blood (UCB), as well as other non-hematopoietic or somatic SCs. SCs in adults are characterizedly considered to be restricted in their regenerative and differentiative potential, while embryonic stem cells are ‘true’ totipotent/pluripotent cells, due to their ability to develop into endoderm, ectoderm, or mesoderm – all three embryonic tissue types in the human body. They are the most promising, but also the most controversial type of potentially transplantable SCs. Immature hematopoietic SCs have the potential of differentiating, not only into all blood cells, but also into some somatic cell types (SC plasticity). In different clinical settings, the transplantation of immature stem cells leads to the repopulation of recipient bone marrow, with subsequent complete, stable, and long-term reconstitution of hematopoiesis. Given that immature stem cells are also capable of homing to different tissues, autologous stem cell implantation into a damaged and/or ischemic area induces their colonization and consecutive transdiffereniatization into cell lineages of the host organ, including neovascularization. Thus, they are clinically applicable in the field of regenerative medicine for the treatment of myocardial, brain, vascular, liver, pancreatic, and other tissue damage. The purpose of this overview is to recapitulate the key developments in the rapidly evolving area of stem cell research, as well as to review the use of SCs in conventional transplantations and in regenerative medicine. Additionally, a brief critical evaluation of our own stem cell research will be summarized.

Key words: stem cells, SC plasticity, transplantation, regenerative medicine
Multicyclic and permanent cytopoiesis is the process of cell development or expansion of a large number of progenitors and precursors, as well as mature cells, from a small number of undifferentiated (immature) stem cells (SCs), in vivo or ex vivo [1–3]. Generally, SCs guarantee steady-state homeostasis in every tissue-generating system. It has been believed for a long time that only embryonic SCs (ESCs) are totipotent/pluripotent, since SC plasticity is essential in early development. Thus, for a long time, cellular totipotency/pluripotency was considered a property of only specific ESCs. However, the most primitive adult SC compartments also have comparatively ‘limitless’ self-renewal potential, as well as the ability to ‘switch’ into other cell lineages (inter-systemic SC plasticity) [2–6].

During cytopoiesis, a complex system of interactive substances, cytokines, growth factors, and inhibitors is regulated and balanced. A partial or complete loss of balanced control can lead to uncontrolled cell growth or death. Over time, i.e., with ageing, SC activity leads to gradual deterioration of the regenerative potential of cells and tissues and the decline of organ repair/renewal capacity, manifesting as disease and tissue defects, including cancer [2–4].

Hematopoiesis is a dynamic hemobiological process, wherein a large quantity of all the types of blood cells is produced from very primitive hematopoietic SCs (HSCs). HSCs are distributed to different hematopoietic compartments throughout the body, during fetal development and adult life. The bone marrow (BM) of adults, as a primary location, has a high potential of differentiation into pluripotent and committed progenitors, which finally transform into various mature blood cells, necessary for blood turnover and the fight against infections [7–15].

Intrinsic genetic pathways control hematopoiesis. Interactive extrinsic signals from the extracellular matrix (ECM) and other signaling molecular pathways, as well as the stromal cell microenvironment are regulated by basic genetic pathways [2,11–14]. The role of stromal cells, including macrophages, fibroblasts, dendritic, endothelial, and other cells, is to stimulate SCs. Namely, these stromal cells stimulate SCs by producing specific growth factors, such as the Flt3-ligand, c-kit-ligand or SC-factor, as well as interleukins, granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor (GM-CSF and G-CSF). In addition to secreting cytokines, stromal cells regulate the adhesion of SC molecules and allow them to stay in the BM or migrate to the area where the respective cell type is needed [14–19].

Populations of HSCs, also named CD34+ cells, express the CD34 antigen. Namely, CD34 is the name...
Populacije MĆ, označene i kao CD34+ čelije, iskazuju (ekspressija) antigen CD34. Naime, CD34 je ime koje je dato transmembranskom glikoproteinu, prisutan na površini MČ, ali i nekih stromalnih čelija. Adultne čelije koje eksprimuju CD34 antigena, a koje su poreklom iz kostne srži ili perifernih krvi, omogućavaju kompletnu i dugotrajnu repopolaciju kostne srži priamaoca (nakon prihvatanja kalaema), sa sleđevenom rekonstitucijom hematopoze (16–21).

Po pravilu, transplantacija MĆ uključuje intenzivnu radiohemoterapiju (mijeloablacija) uz infuziju prikupljenih čelija, što se sprovodi da bi se postigla eliminacija onovne bolesti i/ili eliminisali određeni poremećaji, kao i da bi se poboljšalo kliničko stanje bolesnika. Kod bolesnika, kod kojih ne može biti primenjena visokodozna radiohemoterapija zbog starosti ili koncombidita, može biti sprovedena procedura kondicioniranja smanjenog intenziteta (engl. reduced-intensity conditioning – RIC) (1–3,8,9). Kako bi mogle da se daju bolesniku odmah nakon prikupljanja ili nakon dugoročnog čuvanja u zamrznutom stanju – krioprezervacija (2–5), MĆ se mogu prikupiti na tri načina (8,17–22):

- a) višestrukom aspiracijom iz kostne srži;
- b) prikupljanjem iz perifernih krvi (engl. peripheral blood – PB) nakon mobilizacije hemoterapijom i/ili faktorima rasta (rHuG-CSF);
- c) izdvajanjem iz krvi pupčane vrpce (engl. umbilical cord blood – UCB).

Hematološka oboljenja (uglavnom maligna) kao i određena benigna oboljenja (teška kombinovana imunodeficijencija – TKI, teška aplastična anemija i različita autoimunska ili metabolička oboljenja) do sada su bila najčešća indikacija za transplantaciju MĆ. U današnje vreme, transplantati MĆ prikupljeni iz perifernih krvi ili kostne srži su češći kod adultne alogene ili autologne transplantacije. Transplantatni MĆ izdvojeni iz krvi pupčane vrpce pokazali su ohrabrujuće rezultate u pedijatriji, uglavnom u slučajevima kada nije moguće naći podudarnog nesrodnog davaoca MĆ (2–4,8).

Plastičnost MĆ je fenomen interstemske čelija, koji odlikava široki fenotipski potencijal vrlo primitivnih MĆ, sposobnih za ukalemljenje u različita tkiva tokom implantacije autolognih MĆ na oštećeno mesto, uz sleđevno transdifereentovanje u čeljske linije tkiva/organa domaćina i razvoj terapeutске mikroangiogeneze (neovaskularizacije) podstaknute faktorima angiogeneze (11–15,23–25).

Intenziviranjem transplantacija MĆ i uvođenjem inovativnih oblika regenerativne i restorativne čeljske terapije, došlo je do povećanja potreba za MĆ kao i za praktičnim operativnim procedurama i metodama manipulacije ovih čelija. Stoga, ovaj rad sažima hemobiologiju MĆ i praktične aspekte optimizacije prikupljanja, prečišćavanja i kliničke primene MĆ, given to a transmembrane glycoprotein present on the surface of HSCs and some stromal cells. Adult cells expressing the CD34 antigen, obtained from BM or peripheral blood (PB), stimulate complete and long-term repopulation of the recipient's bone marrow (following engraftment), with subsequent hematopoietic reconstitution (16–21).

Traditionally, SC transplantation involves intensive radiochemotherapy (myeloablation) coupled with infusion of collected cells, which is done in order to achieve the eradication of the underlying disease and/or eliminate certain disorders, as well as improve the clinical status of the patient. Patients who are ineligible for high-dose radio-chemotherapy because of their age or comorbidity, may be offered a similar procedure with reduced-intensity conditioning (RIC) (1–3,8,9). In order to be administered immediately after harvesting or after long-term storage in the frozen state – cryopreservation (2–5), SCs can be collected in three ways (8,17–22):

- a) multiple aspirations from BM;
- b) harvesting from PB after mobilization with chemotherapy and/or growth factors (rHuG-CSF);
- c) processing from umbilical cord blood (UCB).

Hematological diseases (mostly malignant) and certain benign diseases (severe combined immunodeficiency – SCID, severe aplastic anemia, and different autoimmune or metabolic disorders) have thus far been the most common indication for SC transplantation. Nowadays, SC transplants derived from PB and BM are more common in adult allogeneic or autologous transplantation. UCB-derived SC transplantations have achieved encouraging results in the pediatric setting, mainly when a matched unrelated SC donor is not available (2–4,8).

SC plasticity is a phenomenon of inter-systemic cell plasticity, which reflects the wide-ranging phenotypic potential of very primitive SCs, capable of homing to different tissues during the implantation of autologous SCs into the damaged area, with subsequent transdifferentiation into cell lineages of the host tissue/organ and the development of therapeutic micro-angiogenesis i.e., neovascularization stimulated by angiogenesis growth factors (11–15,23–25).

Both the intensification of SC transplantations and the introduction of innovative cell-mediated restorative or regenerative therapy, increase the need for SCs and for practical operating procedures and manipulation methods in relation to these cells. Therefore, this paper summarizes the SC hemobiology and the practical aspects of the optimization of cell harvesting, purification, and clinical use.
EMBRIONALNI NASPRAM ADULTNIH ODELJAKA MATIČNIH ĆELIJA

Odeljci MĆ uključuju populacije čelija koje su karakteristične i za EMĆ i za adultno tkivo. Opšte su izvješću, samo su EMĆ čelije „prave“ totipotentne/pluripotentne čelije, zato što je fenomen intersistemskih plastičnosti MĆ tokom ranog razvoja živih bića od ključnog značaja. U pitanju je sposobnost EMĆ da se razviju u svaku od tri vrste embrionskog tkiva. Nasuprot tome, smatra se da su MĆ kod odraslih osoba po pravilu ograničene, i u regenerativnom potencijalu i u potencijalu diferentiranja, na tkiva koja „nastanjuju“ [6]. Stoga, hepatociti mogu da proliferiraju (i manje-više da se diferentijuju) nakon delimične hepatoktinomije; MCH mogu da rekonstitušu čelije krvi nakon oštećenja kostne srži (usled hemoterapije i/ili zračenja); progenitori keratinocita mogu da učestvuju u zarastanju rana; a određene „sate"teliške" čelije mogu da obnove oštećene skeletne mišiće. Uz ulogu u regeneraciji oštećenih tkiva, MĆ imaju važnu funkciju u održavanju homeostaze tkiva. Na primer, MĆ imaju specifičnu ulogu u održavanju tkivne homeostaze tokom celog života jedinke [2–6].

Embrionalne matične čelije

Kao što je već navedeno, samo su EMĆ validne odnosno „autentične“ totipotentne/pluripotentne MĆ, zbog njihovog potencijala da se diferentiju u bilo koju od čelijkih linija, odnosno njihove sposobnosti da sazru u bilo koji od tri tipa tkiva/germinativnih slojeva u ljudskom organizmu – endoderml, ektoderml ili mezoderml. Nasuprot tome, smatra se da su MĆ kod odraslih osoba, po pravilu, ograničene u svojim razvojnim i regenerativnim potencijalima na tkiva koja „nastanjuju“, uprosodrenim nalazima u današnje vreme koji ukazuju na to da neke adultne MĆ pokažu plastičnost koja je slična biočelijom i endodermu, ektodermu ili mezodermu. Naime, prve primivitne čelije potekle iz kostne srži, kao što su veoma male MĆ našli na embrionalnim (engl. very small embryonic-like SCs – VSELs), takođe imaju sposobnost da se razviju u razne somatske čelije, zahvaljujući već spomenutoj plastičnosti MĆ kojom se odlikuju [2,26–34].

Doba inovacija u oblasti fiziologije embrionskih čelija je započelo krajem prošlog veka izdvajanjem čelija koje poseduju totipotentnost (potencijal da se diferentiju u bilo koju od čelijskih linija u ljudskom telu), iz humanih blastocisti i fetalnog tkiva. Nakon toga, naučnici su opisali važne hemobiološke i molekularne karakteristike ovih čelija i unapredili metode njihove kultivacije [2–6].

Usled najvišeg stepena čelijkih plastičnosti, zgorit se smatra „autentičnom“ totipotentnom/pluripotentnom MĆ. Do kraja petog ili šestog dana deobe, nadalje

EMBRYONIC VERSUS ADULT STEM CELL COMPARTMENTS

SC compartments include cell populations characteristic of both ESCs and adult tissue. Generally, only ESCs are “true” totipotent/pluripotent cells, because the phenomenon of inter-systemic SC plasticity during early development of living organisms is critical. It is the ability of ESCs to develop into all three embryonic tissue types. Conversely, SCs in adults are characterized by being restricted, in their regenerative as well as their differentiative potential, to tissues where they reside [6]. Therefore, hepatocytes can proliferate (and more or less differentiate) following partial hepatectomy; HSCs can reconstitute blood cells after BM damage (due to chemotherapy and/or irradiation); keratinocyte progenitors can participate in wound healing; and certain ‘satellite’ cells can repair injured skeletal muscles. In addition to their role in the regeneration of damaged tissues, SCs have an important function in maintaining tissue homeostasis. For example, SCs have a specific role in maintaining tissue homeostasis throughout the entire life of an individual [2–6].

Embryonic stem cells

As stated above, only ESCs are valid or ‘authentic’ totipotent/pluripotent SCs, due to their potential to differentiate into any of the cell lineages, i.e., their ability to mature into any of the three tissue types/germ layers in the human body – the endoderm, ectoderm, or mesoderm. Conversely, SCs in adults are considered limited in their developmental and regenerative potential, typically to the tissues where they reside, despite the current findings that some adult SCs have SC plasticity, similar to the biological potential of ESCs. Namely, very primitive BM-derived cells, such as very small embryonic-like SCs (VSELs), are also able to develop into a variety of somatic cells, due to the aforementioned SC plasticity [2,26–34].

The age of innovation in ESC physiology began at the end of the last century, with the separation of cells, which possess totipotency (the potential to differentiate into any of the cell lineages in the human body), from human blastocysts and fetal tissue. Subsequently, researchers described the important hemobiological and molecular characteristics of these cells and improved methods for their cultivation [2–6].

Due to the highest degree of cell plasticity, the zygote is considered to be the ‘authentic’ totipotent/pluripotent SC. By the end of the fifth or sixth day of division, it further develops from the older cells of the blastocyte that initiate the expansion of the coding sequence for specific functions, which makes it possible
se razvijaju iz starih ćelija blastocite koje podstiču širenje kodne sekvence za specifične funkcije, čime postaje moguće da se izoluju EMC. Akumulacija fetalnih MČ u fetalnoj jetri čini ove ćelije hipotetički pogodnim za ekstrakciju iz blastocite i kultivaciju ex vivo u MČ. Posle njihove transplantacije bolesnicima, smanjena je verovatnoća odbacivanja aloantrotlata, pošto ove MČ imaju vrlo malo ili nimalo proteina okidača imunskog sistema na svojoj površini [2,3,5].

Uprkos jako etičkoj argumentaciji da se istraživanje nje, a naročito njihova potencijalna klinička primena, graniči sa ubistvom iz nehata [3,5], EMC najviše obećavaju kao ćelije koje se mogu potencijalno pre saditi, od svih ostalih MČ. Naime, bazična istraživanja i neka pretklinička ispitivanja EMC su se pokazala kao značajna u razvoju novih metoda zamene ćelija i strategija za regenerisanje oštećenih tkiva i ponovno uspostavljanje ključnih funkcija u obolelom ljudskom organizmu [2,26–34].

Pojam i funkcionalnost adultnih matičnih ćelija

Adultne MČ identifikovane u raznim tkivima/organima (kostna srž, periferka krv, zubna pulpa, masno tkivo, fetalna jetra, krvi sudovi, mozak, srce, skeletni mišići, koža, gušterača, i gastrointestinalni trakt) obično su zrelije od EMC [2–6]. Ranije se smatralo da su adultne MČ „nesposobne“ da proizvedu ćelijeske linije tri već najvedena tipa tkiva (endoderma, ektoderma i mesoderma) zato što ne mogu da se „podmlade“ (u ranijoj ćelijskoj fazi) procesom diferenciranja ćelija – uz proces transdifferentiranja. Adultne MČ su sposobne da se samoobnavljaju, ali se češće dele kako bi proizvele progenitore i prekursor, kao i zrele ćelije specifičnih ćelijskih linija.

Slika 1 pokazuje da samo kombinacija nekoliko uslova može da posluži kao potvrda da su se neke MČ dobijene iz kostne srži, zaista transformisale u somatske ćelije (ćelije specifične za solidne organe) [2,4,23–25].

MČH su nesumnjivo najbolje proučeni specifični tip MČ kod odraslih osoba koje imaju potencijal da re konstituiju sve ćelije krvi. Ove klase MČH, kratkotrajno (engl. short-term HSCs – ST-HSCs) i dugotrajno kolonijuće ćelije (engl. long-term HSCs – LT-HSCs), mogu da rekonstituišu krv eksperimentalnih životinja – i to, na jedan do dva meseca (ST-HSCs), odnosno na više od šest meseci (LT-HSCs) [2,5]. Nakon uakumlemljenja, MČ dobijene iz kostne srži mogu da prođu proces koji se sastoji iz više faz, uključujući migraciju, konverziju u novi ćelijski fenotip, kao i ispoljavanje funkcija karakterističnih za tkivka koja „nastanjaju“ [4,23,24].

MČ dobijene iz kostne srži su heterogena populacija sa morfofološkim i funkcionalnim karakteristikama tkivno opredeljenih MČ (engl. tissue committed SCs – TCSCs). Moguće je da su zadužene za reparaciju manjih

to isolate ESCs. Accumulation of fetal SCs in the fetal liver makes them hypothetically suitable to be extracted from the blastocyte and to be cultured ex vivo into SCs. After their transplantation into an individual, the possibility of rejection is reduced, since these SCs have little or no immunity-triggering proteins on their cell surface [2,3,5]. Despite strong ethical arguments that ESC research, and especially potential clinical use, border on negligent manslaughter [3,5], ESCs are the most promising potentially transplantable SCs. Namely, basic research and some preclinical investigations with ESCs have proven to be important for the development of new cell-replacement methods and strategies to restore damaged tissues and re-establish critical functions of the diseased human body [2,26–34].

Adult stem cells – concept and functionality

Adult SCs identified in various tissues/organisms (BM, PB, dental pulp, adipose tissue, fetal liver, blood vessels, brain, heart, skeletal muscle, skin, pancreas, and the gastrointestinal tract) are usually more mature than ESCs [2–6]. Previously, adult SCs were considered ‘incapable’ of producing cell lineages of the three above-mentioned tissue types (endoderm, ektoderm and mesoderm) because they could not be ‘rejuvenated’ (in the earlier cell phase) by the process of cell dedifferentiation – accompanied by transdifferentiation. Adult SCs are capable of self-renewal, but divide more frequently to produce progenitors and precursors, as well as mature cells of specific cell lineages.

Figure 1 shows that only the combination of several conditions can be used to confirm that some SCs, derived from BM, have in fact transformed into somatic cells (cells specific to solid organs) [2,4,23–25].

HSCs are undoubtedly the most thoroughly understood specific type of SCs in adults which have the potential to reconstitute all blood cells. Both identified classes of HSCs, short-term HSCs (ST-HSCs) and long-term HSCs (LT-HSCs), can reconstitute the blood of experimental animals for one to two months, and more than 6 months, respectively [2,5]. After homing, BM-derived SCs can undergo a multistep process involving migration, conversion to a new cellular phenotype, and expression of functions characteristic of the tissue where they reside [4,23,24].

BM-derived SCs are a heterogeneous population of cells with morphological and functional characteristics of tissue committed SCs (TCSCs). They may be in charge of healing minor tissue damage; their number among mononuclear cells (MNCs) is very low – approximately one cell per 1,000 to 10,000 total nucleated

MATIČNE ĆELJE – OPŠTI PREGLED: OD RAZVOJNIH HEMOBILOŠKIH KONCEPATA DO (AUTO)GRAFTING-a U KLINičKOJ PRAKSI

A STEM CELL OVERVIEW – FROM EVOLVING HEMOBIOLICAL CONCEPTS TO (AUTO)GRAFTING IN CLINICAL PRACTICE
oštećenja tkiva; njihov broj među mononuklearnim ćelijama (engl. mononuclear cells – MNCs) je veoma nizak – oko jedna ćelija na 1.000 do 10.000 od ukupnog broj ćelija s jedrom (engl. total nucleated cells – TNCs) u kostnoj srži \[2,19\]. Međutim, kod teških oštećenja (infarkt miokarda ili moždani udar), oni imaju mogućnost da ispolje svoj puni terapijski potencijal. Migracija ovih ćelija u oštećene zone zavisi od ‘homing signal’ , koji može biti neefikasan u prisustvu citokina ili prote -
oznih enzima, koje oslobađaju leukociti i/ili makrofa-
gi oštećenog tkiva \[23\]. Ipak, dok su ‘zarobljene’ od-
nosno ‘inkapsulirane’ u kostnoj srži, ove ćelije mogu
biti u posebnom ‘latentnom stadijumu’ – nisu u pot-
punosti funkcionalne, te su im potrebni odgovarajući
aktivacioni signali \[2–4,23\].

Mesenchymal stem cells/stromal cells

Compared to ESCs and induced pluripotent SCs (iP-
SCs), mesenchymal stem cells/stromal cells (MSCs)
have a high-quality therapeutic potential and safety
profile \[2–6\]. The molecular and functional character-
istics of the dental pulp make it an important source
of dental pulp SCs, including adult MSCs \[2,34–38\].
MSCs of ectomesenchymal origin located in the peri-
vascular niche are considered highly proliferative, mul-
tipotent, and similar to BM-derived SCs. Other dental
pulp SCs (DPSCs), stem cells from human exfoliated de-
ciduous teeth (SHED), and immature dental pulp cells
(IDPC) can transdifferentiate into various cells, such as

Slika 1. Pokazuje da samo kombinacija nekoliko uslova može da posluži kao potvrda da su neke MĆ, dobijene iz kostne srži, zaista transformisale u somatske ćelije (ćelije specifične za solidne organe) \[2,4,23–25\]

Figure 1. Shows that only the combination of several conditions can be used to confirm that some SCs, derived from BM, have in fact transformed into somatic cells (cells specific to solid organs) \[2,4,23–25\]
porekla, koje se nalaze u perivaskularnoj niši, visoko proliferativne, multipotentne i slične MČ poteklom iz kostne srži. Druge MČ iz zubne pulpe (engl. *dental pulp SCs* – DPSCs), MČ iz „eksfoliranih“ humanih mlečnih zuba (engl. *stem cells from human exfoliated deciduous teeth* – SHED), kao i primitivne čelije zubne pulpe (engl. *immature dental pulp cells – IDPC*) mogu se transdiferentovati u različite čelije, kao što su odontoblasti, hondročiti, osteoblasti, adipociti, nervne/glijalne čelije, čelije glatkih mišića, čelije skeletnih mišića, i druge čelije. Buduća istraživanja trebalo bi da pruže, uz podatke o MČ zubne pulpe, kompleksne podatke o drugim humanim tkivima kao potencijalnim izvorima MČ odgovornih za razvoj/regeneraciju tkiva.

**Pojam veoma malih matičnih čelija nalik embrionalnim (VSEL čelije)**

Specifični tip čelija, koji je prvobitno izolovan iz kostne srži, liči na EMČ i u stanju je da imitira njihovu sposobnost transdiferentovanja u druge tipove čelija. Stavšiće, ove čelije mogu da se transdiferentiraju u nove čeljske linije iz više od jednog germinativnog sloja – u nervne, čelije srca, gušteraca, kao i čelije drugih tkiva odnosno organa. Retajčak i saradnici su bili prvi koji su identifikovali VSEL čelije, poseban tip čelija koji se ponaša drugačije od ostalih MČ dobijenih iz adultne kostne srži [26–30]. Iako su VSEL čelije u suštini vrlo slične ili iste, po ultrastrukturom i proteinskim markerima, kao i EMČ [2,27–29], one se smatraju „kontaminantima“, koji zapravo doprinose pozitivnom regenerativnom kliničkom ishodu [26–32]. Najzad, postoje podaci koji potvrđuju da VSEL čelije regenerišu, dok mezenhimske MČ podrudaju, obolela reproduktivna tkiva [33]. Međutim, autori ovakvih studija su izneli pretpostavku da implantacija MSC čelija naprosto oslobađa faktore rasta ili specifične citokine koji su od ključnog značaja u procesu diferentovanja MČ „nastanjenih“ u tkivu, u spermatozoide ili jajne čelije [33]. Ovo je zanimljiv koncept u regenerativnoj medicini koji bi trebalo da se ozbiljno razmotri u budućim istraživanjima i pretkliničkim ispitivanjima na ljudima.

**Pojam i primena indukovanih pluripotentnih matičnih čelija (iPSC čelije)**

Izuzetan doprinos istraživanjima i primeni MČ dali su Džon Gurdon i Šinya Yamanaka svojim radom nagrađenim Nobelovom nagradom, 2012. godine, koji je pokazao da, uz primenu ključnih molekula, zrele adultne čelije/fibroblasti mogu da se diferentiraju u pluripotentne MČ i da se transdiferentiraju, odnosno konvertuju u različite zrele čelijeske linije (npr. čelije srčanog mišića) [39]. Iako su ograničene u pluripotentnosti i u stanju da indukuju maligne čelije, ove iPSC čelije predstavljaju odontoblasts, chondrocytes, osteoblasts, adipocytes, neuron/glial cells, smooth muscle cells, skeletal muscle cells, and other cells. Future research should provide us with complex data on many human tissues, in addition to DPSCs, as potential sources of SCs responsible for tissue development/regeneration.

**The concept of very small embryonic-like stem cells (VSELs)**

A specific type of cells, isolated initially from BM, look like ESCs and appear to mimic their ability to transdifferentiate into other cell types. Furthermore, these cells can transdifferentiate into novel cell lineages from more than one germ layer – into nerve, heart, pancreatic and cells of other tissues/organs. Ratajczak et al. were the first to identify VSELs, a special type of SC that acts differently than other SCs derived from adult BM [26–30]. Although VSELs have basically very similar or the same ultrastructure and protein markers as ESCs [2,27–29], they are considered ‘contaminants’, which, in fact, contribute to a positive regenerative clinical outcome [26–32]. Finally, there are data confirming that VSELs regenerate, whereas MSCs rejuvenate diseased reproductive tissues [33]. However, the authors of such studies supposed that MSC implantation may simply release growth factors or specific cytokines critical for tissue-resident SCs to differentiate into sperm cells or ova [33]. This is an interesting concept in regenerative medicine, which should be seriously considered in future research and preclinical studies on humans.

**The concept and application of induced pluripotent stem cells (iPSCs)**

An incredible contribution to SC research and application came from the Nobel prize awarded work of John Gurdon and Shinya Yamanaka (2012), which has shown that, with the use of crucial molecules, mature adult cells/fibroblasts can dedifferentiate into pluripotent SCs and transdifferentiate or convert into different mature cellular lines (e.g., cardiac muscle cells) [39]. Although limited in pluripotency and capable of inducing malignant cells, these iPSCs are a potential source with which clinicians can circumvent certain source-related problems. The future will show their implications and impact on SC therapy.

**STEM CELL CATEGORY AND INFLUENCE**

The traditional primary cell source for SC transplantation is the bone marrow. Approximately 1 – 3% of TNCs in BM express the CD34 antigen. The CD34+ cells have been found in PB, but in an extremely small number in steady-state hematopoiesis – only 0.01 – 0.05% of TNCs [2,19].
For transplantation, the SC quantity is a critical issue. Different populations of TNCs and MNCs (including SCs) can be obtained by multiple aspirations from BM, harvesting from PB after mobilization (chemotherapy and/or rHuG-CSF), and processing from UCB. These cells can be clinically applied (transplanted) immediately after collection and cryopreservation [1–4,10].

When the donor is anesthetized, cells can be collected by multiple aspirations from the posterior (seldom anterior) iliac crests, using the maximal target aspirate volume of up to 15 ml/kg of body mass (kgbm) of the donor. Marrow aspirate must be filtered to remove bone or lipid particles and cell aggregates. Citrate solution and heparin diluted in saline (5,000 IU/500 ml) are used for anticoagulation [2–5].

For ABO incompatible SC transplantations, red blood cell (RBC) count and plasma quantity reduction is required (by aspirate processing). Depletion of T-cells is achieved by ex vivo purging (positive or negative immune-magnetic cell selection). Procedures of processing and purging SCs enable the reduction of RBC total quantity by 90% as well as T-cell depletion by 3 – 4 Log₁₀ [2–5].

Nowadays, the number of patients treated with PB-derived SCs is constantly growing, and PB-derived SCs are used for about 80% of allogeneic and practically for all autologous transplants. The characteristics of SC transplantations are the following [2–5,19]:

a) minimally invasive cell harvesting method and absence of general anesthesia risks;

b) small harvesting volume (250 ml) with a better yield of CD34⁺;

c) high engraftment rate (rapid hematopoietic reconstruction) and low transplant-related morbidity (TRM).

Regarding the timing of SC cell harvesting, the CD34⁺ cell count in the circulation is maximal, i.e., it peaks on the 5th day of rHuG-CSF administration (5 – 10 μg/kgbm per day for allogeneic donors). However, the optimal timing of autologous SC harvesting is more complex, and these patients receive a higher rHuG-CSF dose (12 – 16 μg/kgbm or more, daily) combined with chemotherapy [2–5]. It is important that the count of circulating CD34⁺ cells should correlate with a high CD34⁺ yield in the graft. It is estimated that when the number of CD34⁺ cells in PB is higher than 40/μl, the possibility of obtaining CD34⁺ ≥ 2.5x10⁶ per kgbm in the recipient is 60%, after performing one large-volume leukapheresis (LVL) [2–5,21].

An innovative harvesting protocol uses plerixafor (mozobil) to obtain a sufficient SC quantity from ‘poor-mobilizers’. The stromal derived factor 1 (SDF-1) retains SCs in the BM due to its physiological interaction.
petog dana nakon primene rHuG-CSF (5 – 10 μg/kgbm, na dan, za alogene davaoce). Međutim, planiranje optimalnog vremena autolognog prikupljanja MC je ipak kompleksnije, te ovi bolesnici primaju višu dozu rHuG-CSF (12 – 16 μg/kgbm ili više, na dan) u kombinaciji sa hemoterapijom [2–5]. Važno je da ukupan broj CD34+ čelija u cirkulaciji korelira sa visokim brojem prikupljenih CD34+ čelija u graftu. Procjenjuje se da, kada je broj CD34+ čelija u kostnoj srži viši od 40/μl, mogućnost dobijanja CD34+≥2,5x10^6 po kg bm kod primacu iznosi 60%, nakon leukapareze velikog volumena (engl. large-volume leukapheresis – LVL) [2–5,21].

Jedan inovativni protokol prikupljanja čelija podrazumeva upotrebu plerixafora (mozobilja) kako bi se dobila dovoljna količina MC od „loših mobilizera”. Faktor poreklo iz stromal derived factor 1 – SDF-1, zadržava MC u kostnoj srži uslovljeno fiziološke interakcije sa hemokinskim receptorom CXCR. Plerixafor je potentan antagonist alfa hemokin-skog receptora CXCR4, jer uspešno inhibira interakciju CXCR4 – SDF-1. Postoje podaci o unapređenom režimu mobilizacije koji primjenjuje plerixafor sa rHuG-CSF, zbog većeg broja nezrelih MC u cirkulaciji i većeg broja prikupljenih CD34+ čelija u graftu [2–4,20]. Uspešna transplantacija se može očekivati kada se postigne cilj: CD34+ čelija ≥2-4x10^6/kgbm kod primaoca. Naša pretrgališta na unapređenom režimu mobilizacije koji primjenjuje plerixafor sa rHuG-CSF, ukazuju na ograničenu dostupnost davao -

1 engl. HLA – human leukocyte antigens

with chemokine receptor CXCR. Plerixafor is a potent antagonist of the alpha chemokine receptor CXCR4 as it effectively inhibits CXCR4 – SDF-1 interaction. There are data on an improved mobilizing regimen which uses plerixafor with rHuG-CSF on account of a higher immature SC count in the circulation and a high CD34+ yield in the graft [2–4,20]. A successful transplantation can be expected when the target: CD34+ cells ≥ 2–4x10^6/kgbm of the recipient, is achieved. Our preclinical investigation has confirmed that the relative frequency of the very primitive SC subset (CD34+/CD90+) in circulation could be a practical and potentially objective mobilization predictive factor for optimized timing of cell harvesting and a predictor of the harvest quality [1–5,20].

A possibility ≤ 30% (for related donors) and ≤ 85% (for unrelated donors) of finding an adult allogeneic donor, indicates limited donor availability [2–5,18]. In the previous decades, UCB has been used as a viable alternative source of 1HLA-typed (matching) SCs for al-logeneic transplantation [2–5,17].

Neonatal SCs are obtained from UCB, immediately after birth, by a painless and non-invasive method of collection. These cells are less mature than those in BM. The ‘naive’ nature of UCB lymphocytes allows the use of partially HLA-mismatched SC grafts without an increased risk of severe graft versus host disease (GvHD), as compared to BM transplantation from a completely matched unrelated donor [2–5,17]. Due to a limited number of SCs, the UCB is a cell source accepted for pediatric patients and patients for whom a matched unrelated SC donor is unavailable. Unfortunately, children weighing ≥ 45 kg bm have a higher risk of graft failure [2–5,17].

More recently, SC transplantation from haploiden-tical donors for the treatment of patients who do not have HLA-matched donors has continued to increase [40,41].

**CLINICAL PRACTICE: STEM CELL TRANSPLANTATIONS VERSUS REGENERATIVE MEDICINE**

The standard treatment for hematological malignan-cies and certain benign disorders is the infusion of al-logeneic or autologous SCs, collected for the hemato-poietic and clinical recovery of patients after high-dose radio-chemotherapy. A similar procedure with RIC may be offered to patients who are ineligible for high dose conditioning due to age or comorbidity. The idea of treating immune-mediated diseases, such as multiple sclerosis and immune-mediated enteropathy, by using SC transplants, is based on the principle that immuno-noablative treatment can destroy the patient’s anti-self lymphocytes [2–5].

1 HLA – human leukocyte antigens
Nažalost, kod dece čija je težina ≥ 45 kgbm postoji veći rizik da dođe do neprihvatanja kalema [2–5,17].

U novije vreme, u porastu je transplantacija MČ dobijenih od haploidentičnih davaoca za lečenje bolesnika koji nemaju HLA bijenih od haploidentičnih davaoca za lečenje bolesti [2–5,17].

Nažalost, kod dece čija je težina postoji veći GvHD fenomena kalem protiv leukemije (engl. graft-versus-host disease – GvHD) [2–5]. Osim toga, infuzija donor specifičnih limfocita (engl. donor-specific lymphocyte infusion – DLI) efikasna bila je za uspostavu istoimene limfocite [2–5].

Tabela 1 prikazuje najčešće indikacije za transplantaciju MČ, kao što su hematološka bolesti, stadijuma bolesti, oboljenja i od uzrasta bolesnika, njegovog opšteg zdravstvenog stanja, te stečenih bolesti na principu da imunoablativna terapija može uništiti imunska enteropatija transplantacijom MČ, zasnovan od principa da imunoablativna terapija može uništiti limfocite, teškim oblikom aplastične anemije i multiplom nadalnim neseminomskim tumorom germinativnih čelija, kao i bolesnika sa pojedinim karcinoma, extraglandalnim ne-seminal germ cell tumors, severe aplastic anemia, and severe multiple sclerosis [2–5,20–22].

However, specific clinical aspects of SC transplantation in the treatment of various hematopoietical and other disorders, such as optimal transplantation of HLA-matched and affective donors [40,41].
transplantation of MČ). Uz to, uveden je originalni in vitro test, označen kao „Test mešanih progenitora” radi predviđanja kliničkog ishoda terapije primenom DLI [2,5,42]. Iako su TRM i mortalitet radikalno smanjeni, transplantacije MČ mogu rezultovati brojnim komplikacijama, uključujući i one najčešće – neuspešno prihvatanje kalaema, virusne ili optužniške infekcije, te akutni ili hronični GvHD [1–5].

U oblasti regenerativne medicine, intersistemska plasticnost MČ opravdava upotrebu primitivnih MČ u lečenju bolesnika sa oštećenjima srca, jetre, pankreasa i drugih organa ili tkiva. Rezultati kliničkih studija ukazuju na transdiferentovanje MČ u kardiomioцитe nakon primene i ukakljenjenja (homing) u oštećenoj, odnosno ishemijoskoj zoni miokarda. Kosmata srž je najčešće korišćen izvor ćelija koje se koriste za postizanje kliničkog oporavka miokarda zbog sadržaja mešavine različitih populacija progenitora – uključujući MČ, ali i brojne ne-hematopoetske ćelije (npr. TCSC, MSC i VSEL ćelija), koje mogu da se transdiferentuju u različite ćelijске linije [14,23,43–45].

Naš Centar za regenerativnu medicinu počeo je sa intrakoronarnom aplikacijom (u kardiologiji) i intramio- kardnom implantacijom (u kardiohirurgiji) mononukle- snih ćelija (sa prisutnim MČ – MNC/MČ) 2003, odnosno 2006. godine [14,23–25]. Preliminarni rezultati pokazali su da je lečenje akutnog infarkta miokarda sa ST elevacijom (engl. ST-elevation myocardial infarction – STEMI) pomučno intrakoronarne injekcije (petog dana od infarkta) autolognih MNC/SC-a, prikupljenih iz aktivirane kostne srži (kostna srž aktivirana pomoću G-CSF-a u jednoj dozi; 3 – 5 µg/kgbm), bilo delotvorno i bezbedno. Ejekcionalna frakcija leve komore (engl. left ventricular ejection fraction – LVEF) se popravila (povećanje = 5,5 ± 6,6%; četvoro-mesečni period praćenja), a veličina infarktne zone (engl. infarction size – IS) se smanjila (smanjenje = 6,2 ± 5,0%). Dugoročni pozitivni LVEF/IS efekti su bili umereni. Nisu registrovani kardiokvascularni mortalitet, ponovljeni infarkt, klinički manifestna srčana infarctna, maligna aritmija ili drugi neželjeni događaji [14,23,24].

Naši prvobitni rezultati u domenu hirurške revak- skularizacije srca ili koronarne bajpas hirurgije (engl. coronary artery bypass grafting – CABG) udružene intramio-kardnom implantacijom MNC/MČ (grupa: CABG plus MNC/MČ) pokazali su nedvosmislenu superior- nost ove metode. LVEF se značajno popravila (povećanje = 5,0 ± 4,2) kod ovih bolesnika. Takođe je potvrđen značajno poboljšani funkcionalni kapacitet (p < 0,001) pomoću šestominutnog testa hodanja (engl. 6-minute walk test – 6-MWT); (6-MWT; šestomesečni pe- riod praćenja; p < 0,001) u grupi CABG plus MNC/MČ naspram kontrolne grupe (samo CABG). Ova terapija takođe pokazala je bezbedan terapijski pristup timing, therapeutic efficacy, and complications, are not described and discussed in this paper in detail. In brief, the efficacy of SC transplantations depends on the type of disease, its stage, its sensitivity to chemotherapy, the patient’s age, and their general health status, as well as the degree of HLA-matching [2–4,20–22].

The well-known beneficial effects of allogeneic SC transplantation in hemato-oncology have only recently been applied in designing a system that separates the positive effects of graft versus leukemia (GvL) from the negative effects of graft versus host disease (GvHD). In addition to the already known favorable results in the treatment of patients with multiple myeloma and leukemia [2,42], we have also found donor-specific lymphocyte infusion (DLI) to be effective in patients with Philadelphia chromosome-positive CML (relapsed after SC transplantation), and we have introduced an original in vitro test, the so called ’Test of Mixed Progenitors’, to predict the clinical outcome of DLI treatment [2,5,42]. Although TRM and mortality have been radically reduced, SC transplantations can involve a number of complications, including the most common ones – engraftment failure, viral or opportunis- tic infections, and acute or chronic GvHD [1–5].

In the field of regenerative medicine, intersystemic SC plasticity provides a rationale for the use of immature SCs in the treatment of patients with damage to the heart, liver, pancreas, and other organs/tissues. Findings from clinical studies indicate transdifferentiation in cardiomycocytes after the administration and homing of SCs in the damaged or ischemic region of the myocardium. BM was the most frequently used source of cells for clinical cardiac repair, due to its complex mixture of progenitors, including HSCs, but also different non-hematopoietic cells (e.g., TCSCs, MSCs and VSELs) which can transdifferentiate into various cell lineages [14,23,43–45].

Our Center of Regenerative Medicine began applying intracoronary injection (cardiology) and intramyocardial implantation (cardiac surgery) of MNC/SCs in 2003 and 2006, respectively [14,23–25]. Preliminary results have shown that treatment of acute ST-elevation myocardial infarction (STEMI) with intracoronary injection (on the 5th day upon infarction) of autologous MNC/SCs, collected from primed/activated BM (BM activated by G-CSF, single dose; 3 – 5 µg/kgbm), was effective and safe. The left ventricular ejection fraction (LVEF) improved (increase = 5.5 ± 6.6%; 4-month follow-up period) and the infarction size (IS) decreased (decrease = 6.2 ± 5.0%). The long-term positive LVEF/IS effects were moderate. No cardiovascular mortality, reinfarction, clinically manifest heart failure, malignant arrhythmia, or other adverse events were detected [14,23,24].
Balint B. i sar.

ZAKLJUČAK
Transplantacija MČ je, bez sumnje, veoma efikasan modalitet lečenja hematoloških maligniteta i određenih bolesti nestalih zbog poremećaja imunskog sistema. Intenziviranje kliničke upotrebe terapije ćelijama (transplantacija MČ i regenerativna medicina) povećala je potrebu za većim kvantitetom i boljom kvalitetom suspenzije MČ (veći prinos prikupljenih ćelija i bolja vijabilnost). Unapređeni protokoli prikupljanja, prečišćavanja i kriokonzervacije, kao i poboljšane extrakorporalne operativne procedure, svele su na minimum mehanička i termička oštećenja tokom ex vivo manipulacije ćelijama. Neophodna je raspolagati optimizovanim procedurama ekstrakorporalne manipulacije MČ. Primena koncepta plastičnosti MČ podstakla je sve širu terapijsku primenu primitivnih MČ za obnavljanje, odnosno regeneraciju organa. Neophodna su buduća bazična istraživanja, kao i brojne randomizovane kontrolisane velike kliničke studije u oblastima transplantacijske i regenerativne primene MČ, radi bolje procene efikasnosti ove revolucionarne terapije u lečenju ishemijske bolesti srca, ali i oštećenja drugih tkiva, odnosno organa.

KRATAK PREGLED NAJVAŽNIJIH TEMA
Primena konvencionalnih transplantacija MČ je, bez sumnje, delotvoran oblik lečenja hematoloških bolesnika. Opšte poznati efekti alogene transplantacija u hematološkim bolestima, odnosno organa, u lečenju ishemijske bolesti srca, ali i oštećenja drugih tkiva, odnosno organa.

Sukob interesa: Nije prijavljen.

Our initial results in coronary artery bypass grafting (CABG) followed by MNC/SC intramyocardial implantation (the CABG plus MNC/SC group) showed the undoubted superiority of this method. LVEF significantly improved (increase = 5.0 ± 4.2) in these patients. Also, a significantly improved functional capacity (p < 0.001) was confirmed with the 6-minute walk test (6-MWT; 6-months follow-up period; p < 0.001) in the CABG plus MNC/SC versus the control (CABG alone) group. This treatment also proved to be a safe therapeutic approach – cardiovascular mortality was significantly lower (5-year follow-up period; p = 0.049) in CABG plus MNC/SC patients [2,25,45].

CONCLUSION
SC transplantation is undoubtedly an effective treatment for hematological malignancies and certain immune-mediated disorders. The intensification of the clinical use of cell-mediated therapies (SC transplantation and regenerative medicine) has increased the need for a higher quantity/quality of SCs (better cell yields and viability). Improved SC collection, purification and cryopreservation protocols, as well as improved extracorporeal operating procedures, have minimized mechanical and thermal cell damage during ex vivo manipulations. However, it is necessary to have optimized SC extracorporeal manipulations. The implementation of the concept of SC plasticity has stimulated the ever-increasing therapeutic application of immature SCs for organ regeneration/repair. We still need much more fundamental research and a greater number of randomized, controlled and larger clinical trials of SC transplantations, especially in the field of regenerative medicine, in order to investigate the possible role of this revolutionary therapy in treating ischemic heart disease and other tissue/organ damage.

HIGHLIGHTS
The use of conventional SC transplantations is undoubtedly effective in the treatment of hematological patients. The well-known beneficial effects of allogeneic SC transplantations in hematolo- oncology have only recently been used to design a system that separates the positive effects of GvL from the negative effects of GvHD.

SC plasticity is a phenomenon of inter-systemic cell plasticity, which represents a wide-ranging phenotypic potential of very primitive SCs capable of homing to different tissues following implantation into a damaged area, with a subsequent transdifferentiation into the cell lineages of the host tissue/organ.

Intersystemic SC plasticity provides a rationale for the use of immature SCs in the treatment of patients with damage to the heart, liver, pancreas, and other organs/tissues.

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