CLAIMS

1. Method for the isolation of human cardiac progenitor cells subpopulations, comprising the following steps:

a) mincing a heart tissue sample containing a population of cardiac cells;

b) 4-times progressive digestion of the cardiac tissue with an enzymatic mixture until a cell suspension is achieved and filtration is achieved through filters of size comprised between 30 μM and 100 μM;

c) further digestion of residual cardiac tissue obtained in step b) for 16 hours with an enzymatic mixture until a cell suspension is achieved and filtration through filters of size comprised between 30 μM and 100 μM;

d) culturing the cell suspension obtained in step b) and c) in a culture medium suitable to propagate the cardiac cells;

e) preliminary expansion of the cardiac cells obtained in step d) in a culture medium suitable to expand the cardiac cells through removal of the cardiac cells from the culture medium in step d) using an enzymatic or non enzymatic solution;

f) secondary expansion of the cardiac cells obtained in step e) in a culture medium suitable to further expand the cardiac cells through removal of the cardiac cells from the culture medium in step e) using an enzymatic or non enzymatic solution;

g) isolating one or more subpopulations of cardiac cells by positive and/or negative selection by the use of monoclonal antibodies directed against one or more surface antigens expressed in the starting population of cardiac progenitor cells.

2. Method for the isolation of human cardiac progenitor cell subpopulations according to claim 1, wherein said heart tissue sample of step a) is selected from the group consisting of right auricle, left auricle, septum, apex or ventricular biopsy.

3. Method for the isolation of human cardiac progenitor cell subpopulations according to anyone of the claims 1-2, wherein the mincing of step a) occurs in

manual or automatic mode.

4. Method for the isolation of human cardiac progenitor cell subpopulations according to anyone of the claims 1-3, wherein said enzymatic mixture of step b) and c) comprises a basal medium and an enzymatic solution.

5. Method for the isolation of human cardiac progenitor cells subpopulations according to claim 4, wherein said enzymatic solution comprises a mixture of collagenases and/or proteases.

6. Method for the isolation of human cardiac progenitor cells subpopulations according to claim 5, wherein said collagenase and/or protease mixture comprises one or more enzymes selected from the group consisting of collagenase I, collagenase II, collagenase IV, trypsin, EDTA, Accutase™, collagenase A, Dispase™ and Liberase™.

7. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 4-6, wherein said basal medium is Ham's/F12.

8. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 5 or 6, wherein said collagenase mixture is present in a concentration comprised between 0.1 and 3 mg/ml.

9. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 1-8, wherein said medium suitable for multiplying and expanding the cardiac cells of steps d), e) and f) is Ham's/F12 medium supplemented with 10% fetal bovine serum, 2 mM L-glutathione and 5 x 10^{-3} U/ml human erythropoietin, 10 ng/ml of basic fibroblast growth factor and antibiotics.

10. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 1-9, wherein the enzymatic or non enzymatic solution of step e) and f) for cell expansion is selected from the group consisting of Tryple™ Select, a mixture of trypsin and/or EDTA, Cell Stripper™, Accutase™, or Dispase™.

11. Method for the isolation of human cardiac progenitor cells subpopulations

according to anyone of the claims 1-10, wherein said monoclonal antibodies of step g) are marked with biotin or a fluorescent molecule, preferably selected between FITC or APC.

12. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 1-11, wherein said antibodies of step g) are monoclonal antibodies against one or more antigens selected from the group consisting of:

CD1a	CD1b	CD1c	CD1d	CD2
CD3	CD4	CD5	CD6	CD7
CD8a	CD9	CD10	CD11a	CD11b
CD11b activated	CD11c	CD13	CD14	CD15 (SSEA-1)
CD16	CD18	CD19	CD20	CD21
CD22	CD23	CD24	CD25	CD26
CD27	CD28	CD29	CD30	CD31
CD32	CD33	CD34	CD35	CD36
CD38	CD39	CD40	CD41	CD42b
CD43	CD44	CD45	CD45RA	CD45RB
CD45RO	CD46	CD47	CD48	CD49a
CD49c	CD49d	CD49e	CD49f	CD50 (ICAM-3)
CD51	CD51/61	CD52	CD53	CD54
CD55	CD56 (NCAM)	CD57	CD58	CD59
CD61	CD62E	CD62L	CD62P (P-Selectin)	CD63
CD64	CD66a/c/e	CD66b	CD69	CD70
CD71	CD73	CD74	CD79b	CD80
CD81	CD82	CD83	CD84	CD85a (ILT5)
CD85d (ILT4)	CD85g (ILT7)	CD85h (ILT1)	CD85j (ILT2)	CD85k (ILT3)
CD86	CD87	CD88	CD89	CD90 (Thy1)
CD93	CD94	CD95	CD96	CD97
CD99	CD100	CD101 (BB27)	CD102	CD103
CD104	CD105	CD106	CD107a (LAMP-1)	CD108
CD109	CD111	CD112 (Nectin-2)	CD114	CD115
CD116	CD117 (c-kit)	CD119 (IFN-g R alpha chain)	CD122	CD123
CD124	CD126 (IL-6 R alpha)	CD127 (IL7 R alpha)	CD129 (IL-9 R)	CD131
CD132	CD134	CD135	CD137 (4-1 BB)	CD137L (4-1 BB Ligand)
CD138	CD140a	CD140b	CD141	CD143
CD143	CD146	CD148	CD150 (SLAM)	CD152
CD154	CD155 (PVR)	CD156c (ADAM10)	CD158a/h	CD158b (KIR2DL2/L3,NKAT2)
CD158d	CD158e1 (KIR3DL1,NKB1)	CD158F	CD161	CD162
CD163	CD164	CD165	CD166	CD167a (DDR1)
CD169	CD170 (Siglec-5)	CD172a (SIRPa)	CD172b (SIRPg)	CD172g (SIRPg)
CD178 (Fas-L)	CD179a	CD179b	CD180 (RP105)	CD181 (CXCR1)
CD182 (CXCR2)	CD183	CD184 (CXCR4)	CD193 (CCR3)	CD195 (CCR5)
CD196	CD197 (CCR7)	CD200 (OX2)	CD200 R	CD201 (EPCR)
CD202b (Tie2/Tek)	CD203c (E-NPP3)	CD205 (DEC-205)	CD206 (MMR)	CD207 (Langerin)
CD209 (DC-SIGN)	CD210 (IL-10 R)	CD213a2	CD215 (IL-15 Ralpha)	CD218a (IL-18R alpha)
CD220	CD221 (IGF-1R)	CD226 (DNAM-1)	CD229 (Ly-9)	CD231 (TALLA)
CD235ab	CD243	CD244 (2B4)	CD245 (p220/240)	CD252 (OX4OL)
CD253 (Trail)	CD254	CD255 (TWEAK)	CD257 (BAFF,BLYS)	CD258 (LIGHT)
CD261 (DR4,TRAIL-R1)	CD262 (DR5,TRAIL-R2)	CD263 (DcR1,TRAIL-R3)	CD266 (Fn14,TWEAK R	CD267 (TACI)
CD268 (BAFF-R,BAFFR)	CD270 (HVEM)	CD271	CD273 (B7-DC,PD-L2)	CD274 (B7-H1,PD-L1)
CD275 (B7-H2,B7-RP1,ICOSL)	CD276	CD277	CD278 (ICOS)	CD279 (PD-1)
CD282 (TLR2)	CD284 (TLR4)	CD286 (TLR6)	CD290	CD294
CD298	CD300e (IREM-2)	CD300F	CD301	CD303
CD304	CD307	CD307d (FcRL4)	CD314 (NKG2D)	CD317
CD318 (CDCP1)	CD319 (CRACC)	CD324 (E-Cadherin)	CD325	CD326 (Ep-CAM)
CD328 (Siglec-7)	CD334 (FGFR4)	CD335 (NKp46)	CD336 (NKp44)	CD337 (NKp30)
CD338 (ABCG2)	CD340 (erbB2_HER-2)	CD344 (Frizzled-4)	CD351	CD352 (NTB-A)
CD354 (TREM-1)	CD355 (CRTAM)	CD357 (GITR)	CD360 (IL-21R)	beta2-microglobulin
BTLA	C3AR	C5L2	CCR10	CLEC12A
CLEC9A	CX3CR1	CXCR7	delta-Opioid Recepto	DLL1
DLL4	DR3 (TRAMP)	EGFR	erbB3/HER-3	FceRIalpha
FcRL6	Galectin-9	GARP (LRRC32)	HLA-A,B,C	HLA-A2
HLA-DQ	HLA-DR	HLA-E	HLA-G	IFN-g R b chain
Ig light chain k	Ig light chain omega	IgD	IgM	IL-28RA
Integrin alpha9beta1	Integrin beta5	Integrin beta7	Jagged 2	LAP
Lymphotoxin beta receptor (L	Mac-2 (Galectin-3)	MAIR-II	MICA/MICB PE	MSC(W3D5)
MSC(W5C5)	MSC(W7C6)	MSC and NPC(W4A5)	MSCA-1(MSC,W8B2)	NKp80
Notch1	Notch2	Notch3	Notch4	NPC(57D2)
Podoplanin	Pre-BCR	PSMA	Siglec-10	Siglec-8
Siglec-9	SSEA-1	SSEA-3	SSEA-4	SSEA-5
TCRg/d	TCR Vbeta13.2	TCR Vbeta23	TCR Vbeta8	TCR Vbeta9
TCR Vdelta2	TCR Vg9	TCR Valpha24-Jalpha18	TCR Valpha7.2	TCR alpha/beta
Tim-1	Tim-3	Tim-4	TLT-2	TRA-1-60R
TRA-1-81	TSLPR (TSLP-R)			

13. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the preceding claims wherein the selection of step g) is carried out by immunomagnetic separation.

14. Method for the isolation of human cardiac progenitor cells subpopulations according to anyone of the claims 11-13, wherein said monoclonal antibodies of step g) are anti-CD117 and/or anti-CD90.

15. Subpopulation of human cardiac progenitor cells from human auricle obtained by the method of isolation according to claim 14 exclusively by negative selection using anti-CD90 monoclonal antibodies, characterized by a surface marker profile $CD90^-$; by expressing mesenchymal markers CD29, CD44, CD73, CD105 and CD200; by not expressing and by lack of expression of CD90, hematopoietic markers CD34, CD45, CD14 and the immune system marker HLA-DR.

16. Subpopulation of human cardiac progenitor cells according to claim 15, for use in the treatment of cardiovascular diseases, alone or in combination with another subpopulation of human cardiac progenitor cells.

17. Subpopulation of human cardiac progenitor cells according to claim 16, for use in cardiac cell and/or tissue transplant, alone or in combination with another subpopulation of human cardiac progenitor cells.