ARİTMİ HASTALARINDA KÖK HÜCRE TEDAVİSİNİN YERİ VAR MI?

İstanbul Girişimsel Kardiyoloji Kursu

17-18 Şubat 2017

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Kardiyolojide Kök Hücre - I

- Eskiden erişkin kalbinin post-mitotik ve terminal diferansiasyona sahip olduğu düşünülürdü.

GÜNÜMÜZ ;

- Uzun süredir miyokard endotel, düz kas ve fibroblastik hücrelerin bölünebildiği bilinmektedir.

N Engl J Med 2002;346:5–15.
Circulation 2002;106:3009-17.

Kardiyolojide Kök Hücre - II

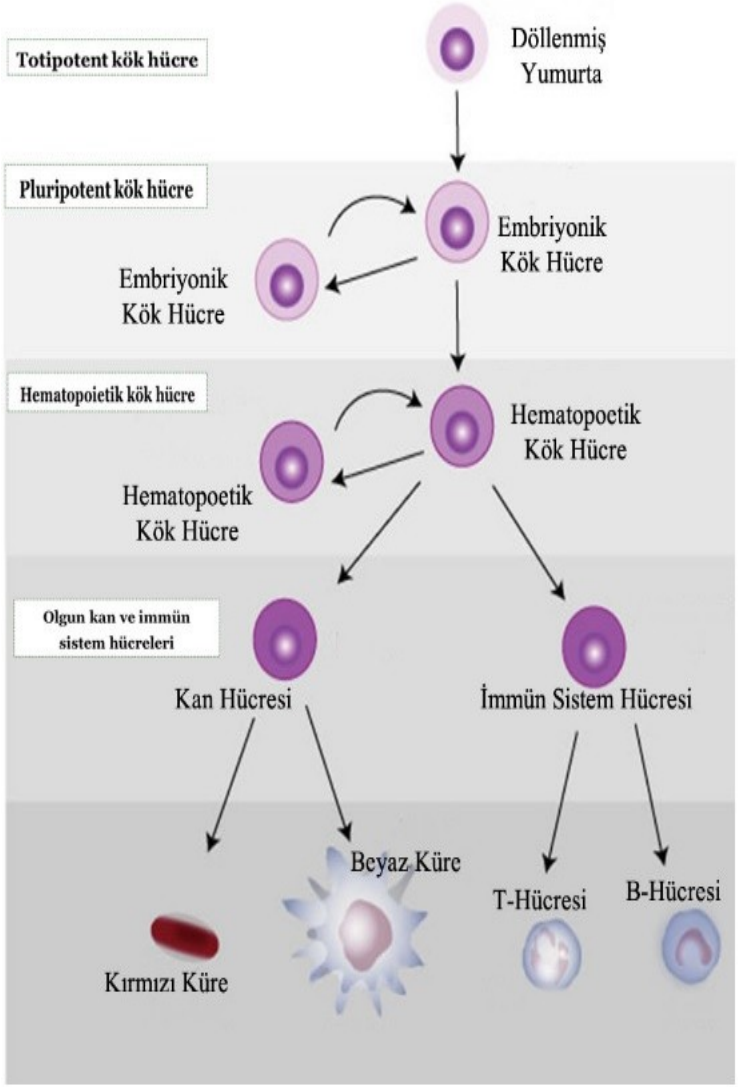
- Bir dokuya ait KH, fonksiyonel olarak farklılaşmamış ve potansiyel olarak heterojen hücrelerdir. Özellikleri:

  - Uygun bir çevreye yerleşme
  - Çoğalma
  - Çok sayıda farklılaşmış yeni hücreler oluşturma
  - Bir hasar sonucunda yeni bir doku oluşturabilme
  - Kendini yedekleme ve idame ettirme

- Kendini yedekleme ve çoğalma kapasitesi embriyonel KH'lerde, erişkin (doku) KH'lerine göre daha iyidir.
- Bu durum ***telomer uzunluğu ve telomeraz aktivitesi*** ile ilgilidir.
- Telomer, kromozomu füzyon ve genetik dengesizliklere karşı korur.

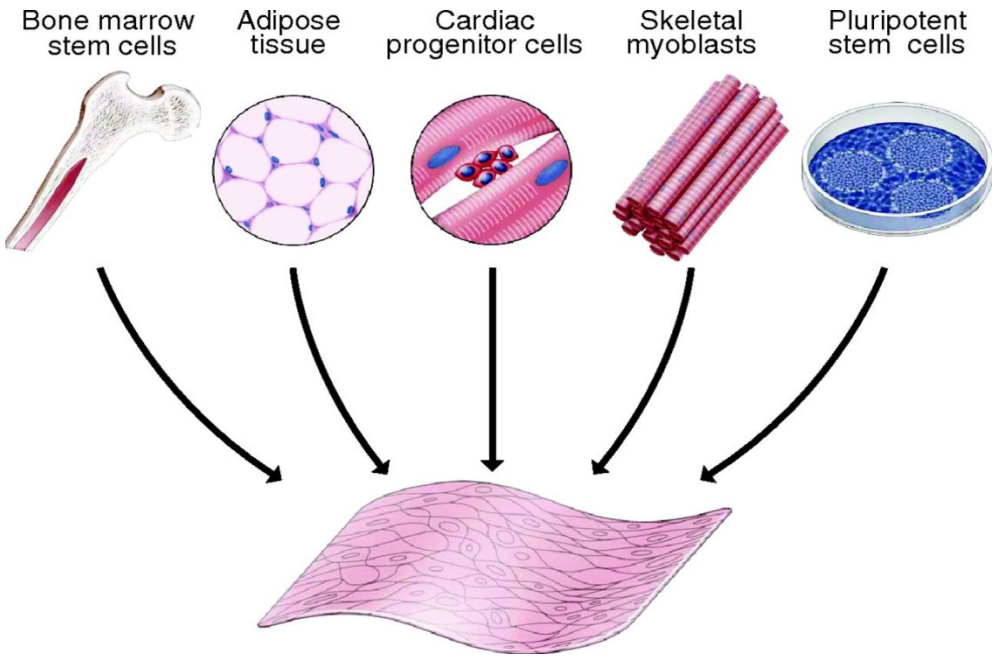
Kardiyolojide Kök Hücre - III

- ***Totipotent KH:*** Tam ve fonksiyon gösteren bir canlıyı meydana getirecek tüm hücre tiplerini oluşturabilirler (erken embriyonel hücreler).
- ***Pluripotent KH:*** Pek çok doku tiplerini oluşturabilirler; fakat fonksiyon gösteren bir canlı oluşturamazlar (embriyonel kök hücre dizinleri).
- ***Multipotent KH:*** Kısıtlı sayıda doku tipi oluşturabilirler (pek çok erişkin KH bu gruptandır).

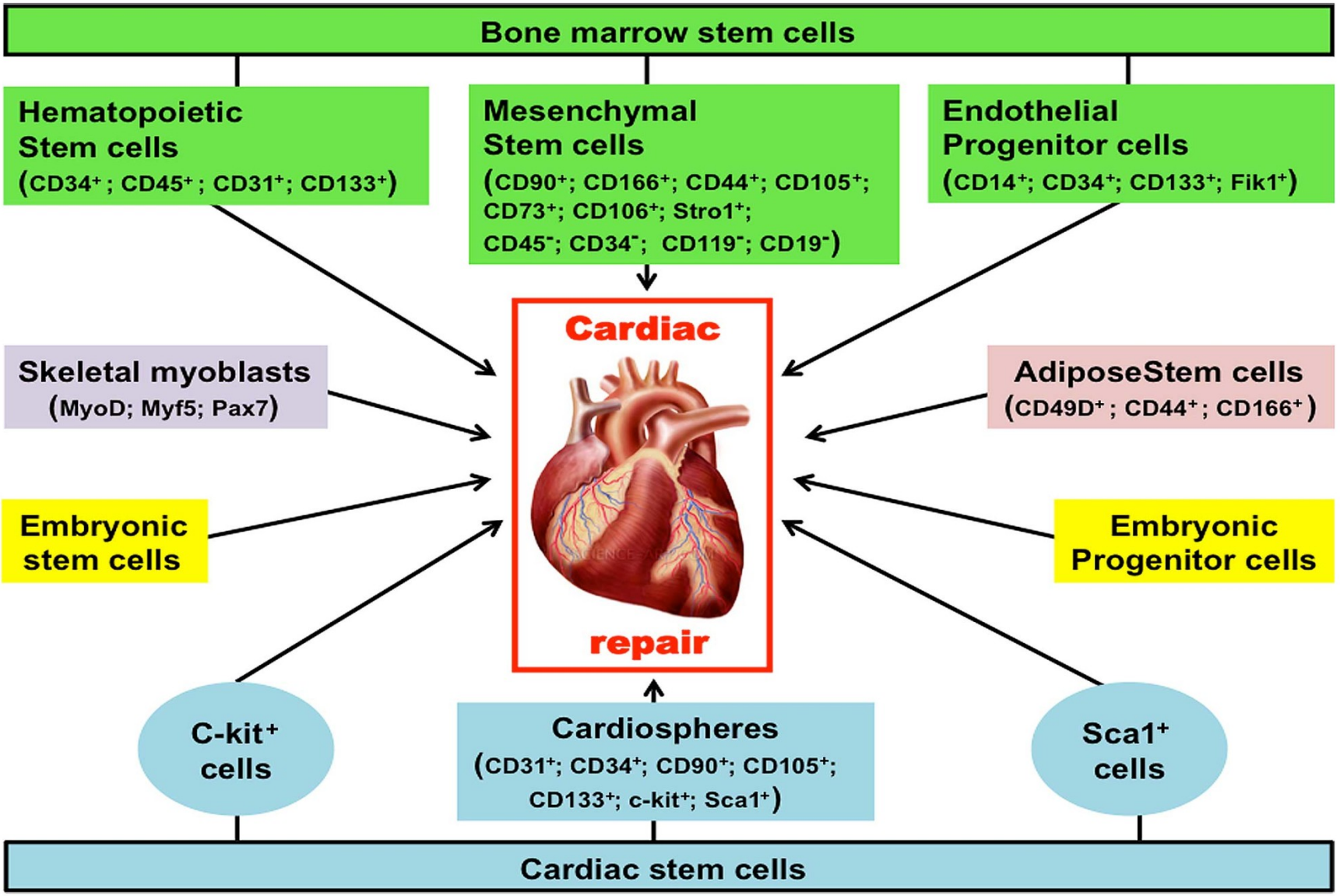


Kardiyak tedavide kullanılan kök hücreler

- Embriyonik kök hücre
- Miyoblast
- Kemik iliği kök hücresi
- Mezenşimal kök hücre
- Endoteliyal progenitör hücre
- Kardiyak prekürsör hücre

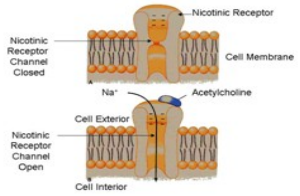


Kardiyak tedavide kullanılan kök hücreler



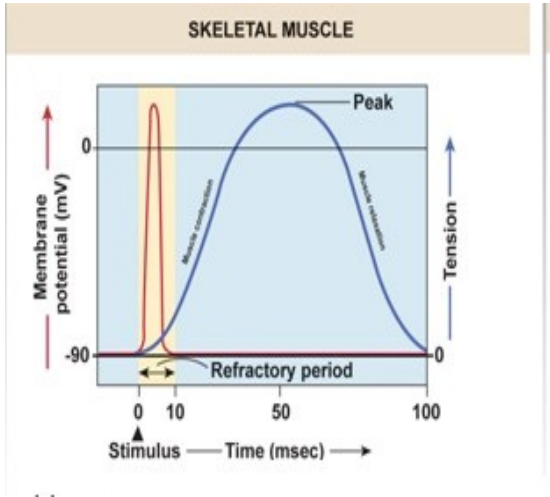
İskelet Miyoblast - I

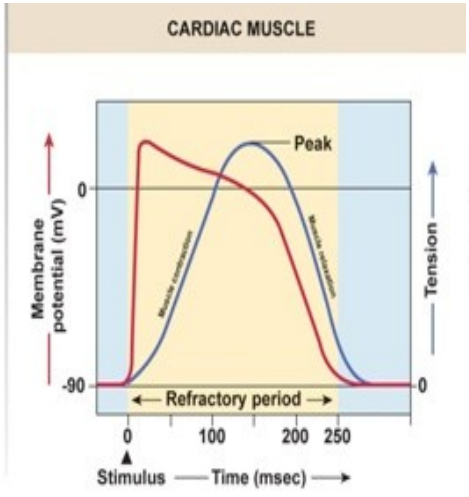
- Kas biyopsisi ile toplanır, kültürde çoğaltılır ve aynı hastanın kalbine enjekte edilir (Otolog).
- İmmünsüpressif ajan gerektirmediğinden tercih sebebi.
- İskemiye dirençlidir.
- Azalmış ileti velositesi ve anatomik engel oluşturarak re-entrant taşikardi riski taşır.

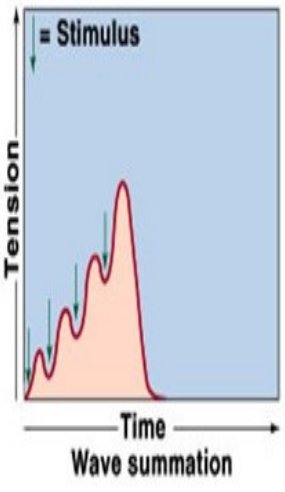


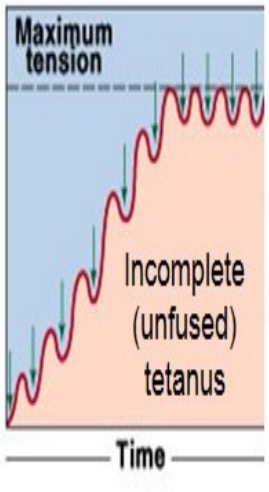
İskelet Miyoblast - II

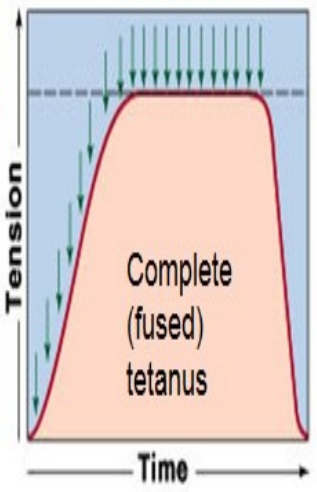
- ***İskelet kası (nikotinik res)'nda AP süresi ve refrakter periyod kardiyomiyositler (muskarinik res)'e oranla çok kısa!***
- Parasempatik uyarı AP'de tetanik aktivasyona neden olarak ICD gerektirebilecek VT'ler oluşturabilir (genelde transplant sonrası 11-30. günlerde).





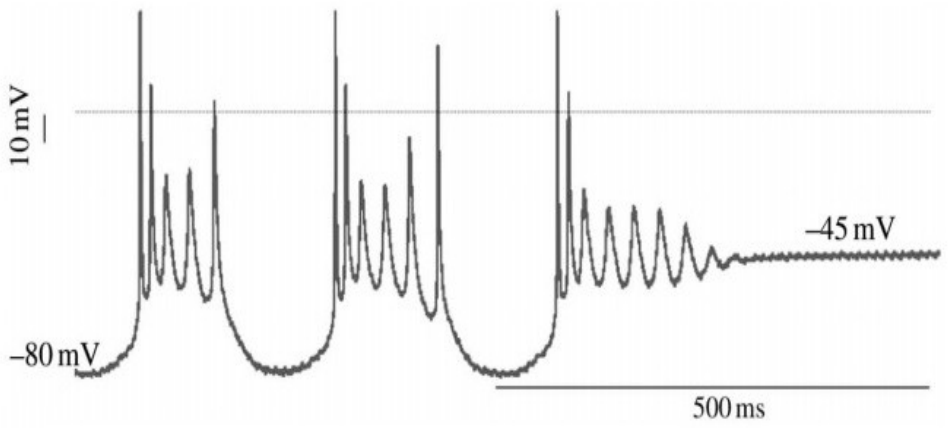






Kemik İliği Kaynaklı Mezenşimal Kök Hücre - I

- Matürasyonun erken döneminde sıklıkla ***unstabil (immatür) AP*** şekli görülür.



- İmmatür miyositler tetiklenmiş aktivite mekanizması ile aritmi oluştururlar (***Deneysel***).

Kemik İliği Kaynaklı Mezenşimal Kök Hücre - II

- Ciddi proaritmik yan etki izlenmemiş: Transplantasyon sonrası neovaskülarizasyonu tetikler, anti-apopitotik faktör salgılatırlar - *Proaritmik etki önleme*

- İnterkale disklere bağlanarak greft ve alıcı arasında sıkı elektriksel bağlantı oluşturur - *İmmatür repol stabilize hale gelebilir*

- Geniş yapısı sebebi ile intrakoroner uygulamalarda, mikrovasküler yatakta oklüzyona sebep olarak iskemiye ve kardiyak aritmilere sebep olabilirler.

Kemik İliği Kaynaklı Hematopoietik Kök Hücre

- CD34 veya CD45 pozitifken; kemik iliği kaynaklı mezenşimal kök hücrelerde negatiftir.
- İn vitro kardiyomiyogeneze rastlanmaz.
- Bozulmuş kardiyak fonksiyonları anjiyogenez ya da parakrin etki ile restore ederler.
- Klinik çalışmalarda herhangi bir proaritmik etki gözlenmemiştir.

Embriyonik Kök Hücre

- Pluripotent, klonojenik ve kendiliğinden yayılan hücrelerdir.
- Farklılaşma safhasında spontan atım gösterir; farklı kalp bölgeleri (atriyum, ventrikül, SAN gibi)'nde değişik AP eğrileri oluşturur.
- Etik ve güvenlik (teratom gibi) problemleri vardır.

STEM CELLS

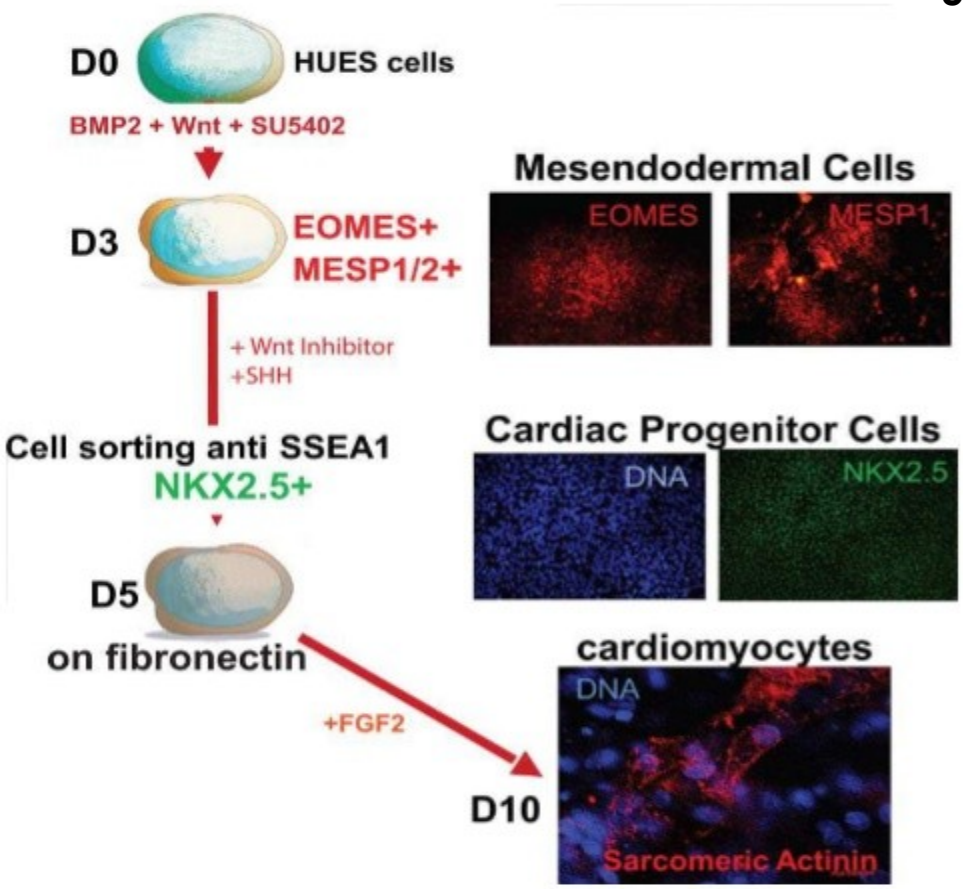
REGENERATIVE MEDICINE

Concise Review: Pluripotent Stem Cell-Derived Cardiac Cells, A Promising Cell Source for Therapy of Heart Failure: Where Do We Stand?

ELODIE GOUADON,[a] THOMAS MOORE-MORRIS,[b] NICOLINE W. SMIT,[c] LUCIENNE CHATENOUD,[d] RUBEN CORONEL,[c] SIAN E. HARDING,[e] PHILIPPE JOURDON,[a] VIRGINIE LAMBERT,[a] CATHERINE RUCKER-MARTIN,[a] MICHEL PUCÉAT[b]

CATHERINE RUCKER-MARTIN,[a] MICHEL PUCÉAT[b]

STEM CELLS 2016;34:34–43



Step by step differentiation of HUES cells into cardiomyocytes. HUES cells are treated with Wnt and BMP2 in the presence of SU5402, a FGFR/VEGFR inhibitor to drive them toward a mesendodermal cell fate (monitored by EOMES and MESP1 expression). Then cells are treated with a Wnt inhibitor, SHH to reach the state of cardiac progenitor cell (NKX2.5+ cells) and sorted with anti-SSEA1 antibody. Cells plated on fibronectin were treated with FGF2 to drive them toward actinin^{+} cardiomyocytes. Abbreviations: HUES cells, human embryonic stem cells; SHH, Sonic Hedgehog.

Kardiyak Progenitör Hücre

- Abcg2 ya da cardiosphare transport proteinlerini eksprese eden c-kit, Sca-1 yüzey markerları ile tanımlanırlar.
- Yüksek etkinlikli kardiyomiyogeneze sahiptirler.

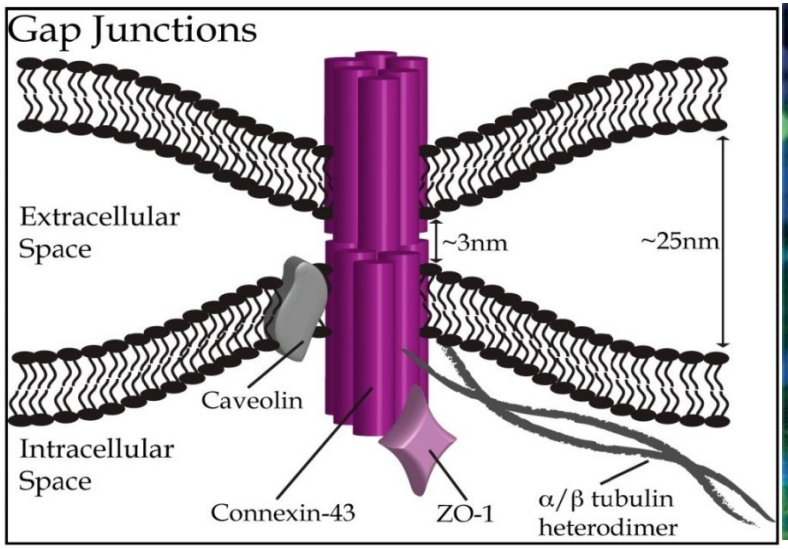
Beltrami A, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763-76.

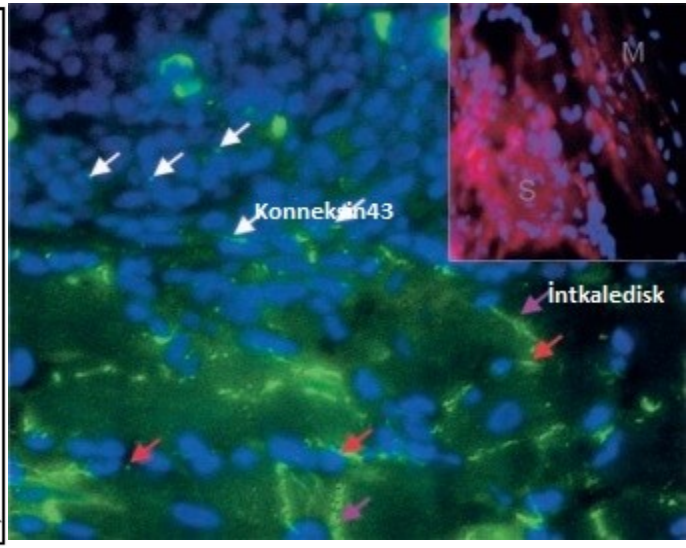
Gap junction

- Hücreler arasında iyon ve küçük moleküllerin geçişine izin veren, membranlarda bulunan, hücrelerarası kanallardır.
- Konneksin'ler tarafından oluşturulurlar.
- Bir hücreden diğerine AP ilerleyişine izin verirler.
- Normal kardiyomiyositlerde, sık olarak interkale disklerde bulunur.

Kanno S, Saffitz JE. The role of myocardial gap junctions in electrical conduction and arrhythmogenesis. Cardiovasc Pathol 2001;10:169-77.

Gap junction





- Değişik kardiyak konneksin izoformları tanımlanmış olup; ventrikül miyositinde konneksin43 en baskın olanıdır.
- Konneksin43 eksikliği ya da yokluğu elektriksel bağlantılarda azalma ve aritmi formasyonu ile ilişkilidir.

de Groot, et al. Conduction slowing by the gap junctional uncoupler carbenoxolone. Cardiovasc Res 2003;60:288-97.

Kök Hücre Nakil Yolları

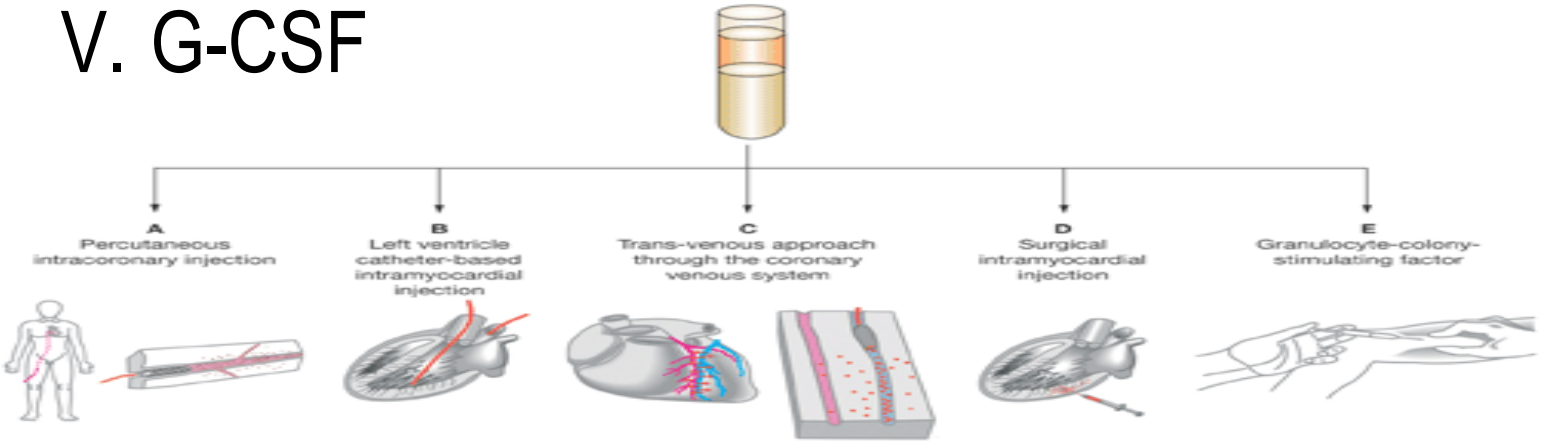
I. Perkütan intrakoroner enjeksiyon

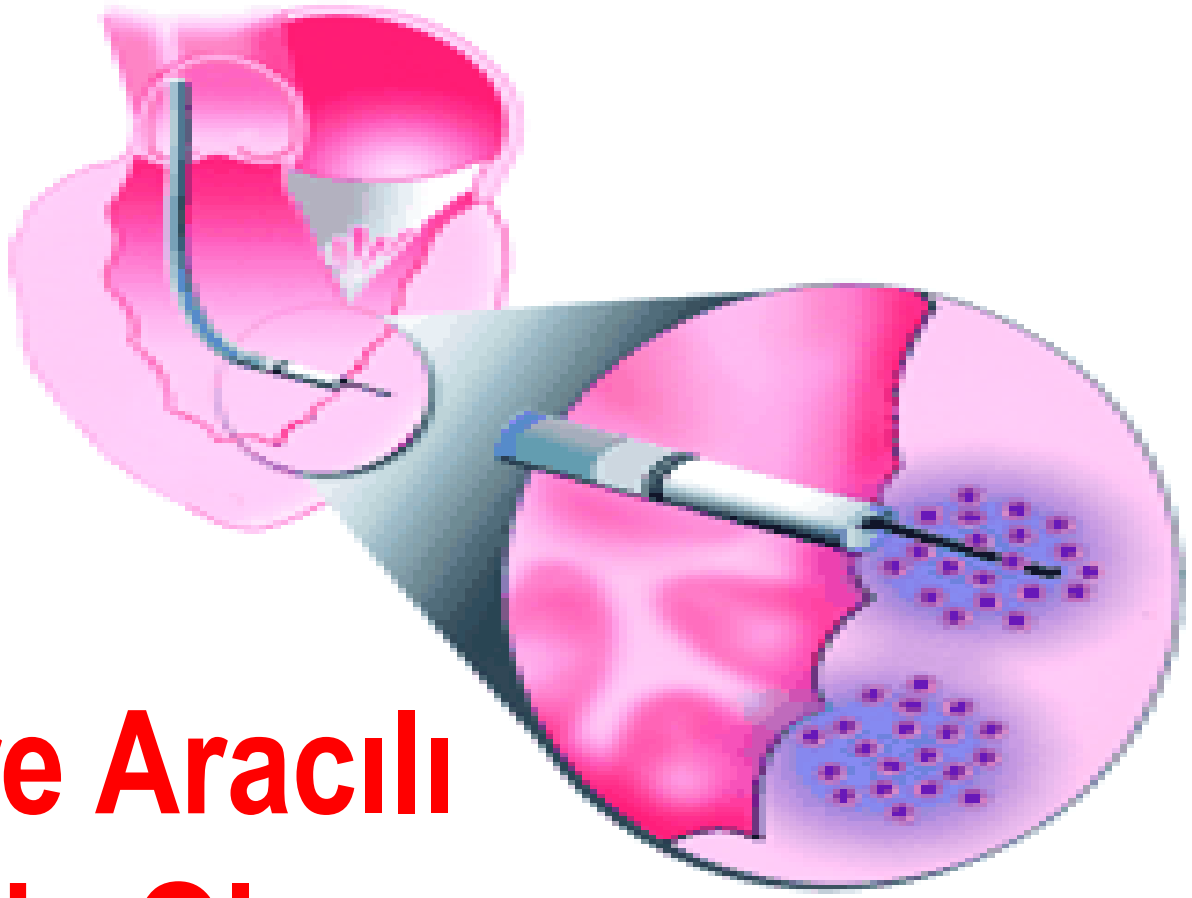
II. Katater ile sol ventrikül intramiyokardiyal enjeksiyon

III. Trans-venöz koroner enjeksiyon

IV. Cerrahi intramiyokardiyal enjeksiyon

V. G-CSF





Kök Hücre Aracılı Aritmilerde Olası Mekanizmalar

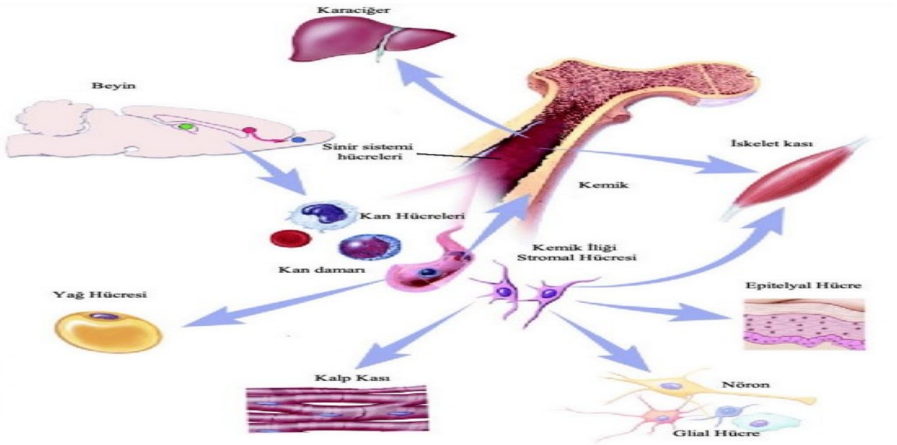
Circ J 2007; **Suppl A:** A-45 – A-49

Cardiac Cell Therapy and Arrhythmias

Shunichiro Miyoshi, MD; Yukinori Ikegami, MD; Yuji Itabashi, MD;
Akira Furuta, MD; Akihiro Umezawa, MD*; Satoshi Ogawa, MD

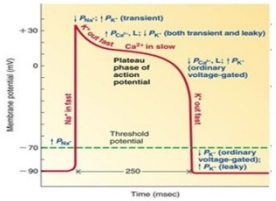
Cardiac stem cell based therapy is a promising therapy for patients with severe heart failure. Many types of stem cells, such as embryonic stem cells, myoblasts, marrow-derived mesenchymal stem cells, circulating endothelial progenitor cells, and cardiac precursor cells etc, are known as cellular sources for cardiac stem cell therapy. Both in the clinical and experimental setting, stem cells are reported, and supposed, to cause some arrhythmogenic adverse effects. In order to overcome these serious adverse effects, it is necessary to know the electrophysiological properties of stem cell-derived cardiomyocytes, and have a profound insight into the mechanisms of arrhythmia to know whether such arrhythmogenic properties of the cells can cause serious arrhythmia in situ. In the present study, recent publications that focus on the electrophysiological aspect of stem cell based therapy are reviewed and, furthermore, a new perspective on cardiac stem cell therapy of arrhythmias is given. (*Circ J* 2007; **Suppl A:** A-45 – A-49)

Key Words: Heart; Proarrhythmia; Stem cell; Sudden death



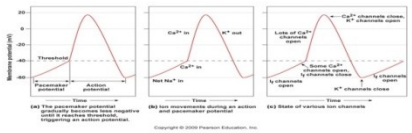
Kök Hücre Aracılı Aritmilerde Olası Mekanizmalar

Miyokard hücresi AP'i

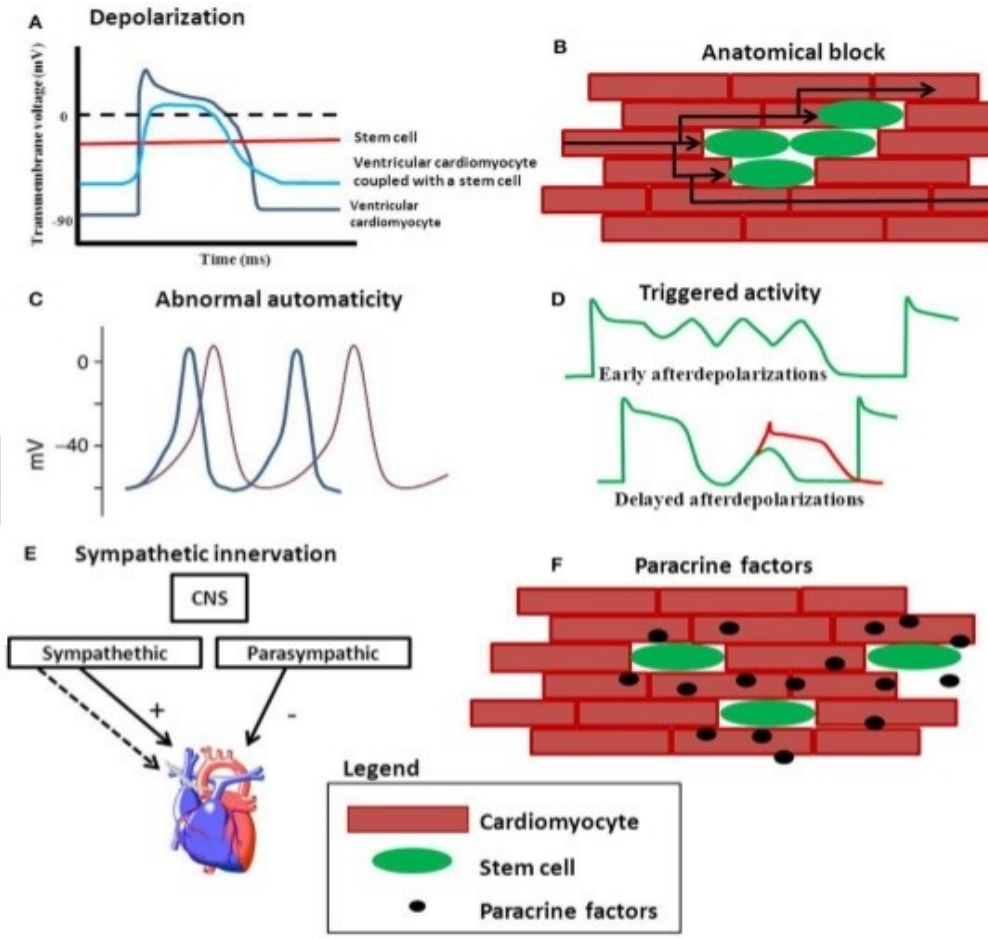


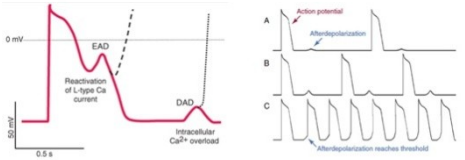
Hızlı depol (Faz 0) - İçe yönelik Na^+ akımına bağlı
Hızlı repol (Faz 1) - Na^+ kanallarının kapanmasına bağlı
Yavaş depol (Faz 2=Plato fazı: 200-300 msn sürer) - İçe yönelik Ca^{+2} akımına bağlı
Repol (Faz 3) - Dışa yönelik K^+ akımına bağlı
İMP(Faz 4)

SAN AP'i



Kararsız bir İMP vardır
Faz 1 ve Faz 2 SAN AP'nde bulunmaz
Faz 0 – AP'nin upstroke'u. İçe yönelik Ca^{+2} akımına bağlı
Faz 3 – Repol. Dışa yönelik K^+ akımına bağlı.
Faz 4 – Yavaş depol. SAN'un önder odak olmasından sorumlu (otomatisite).





AP'in repolarizasyon fazı sonrasında görülen pozitif potansiyellerdir.

Erken AD: Faz 2 ve Faz 3.

Geç AD: Faz 4. Aritmi oluşumunda önemli.

FIGURE 1 | Possible mechanisms of stem cell induced arrhythmias. **(A)** Depolarization of cardiomyocytes reduces the upstroke velocity and conduction velocity. **(B)** Clusters of stem cells can create an anatomical block and force the electrical pathway to find a different (and longer) route. **(C)** Stem cells can be spontaneously beating and may compete with the hosts own automaticity when engrafted. Stem cells may also be capable of inducing arrhythmias via triggered activity **(D)**. **(E)** Increased sympathetic innervation induced by stem cells can give an unbalance in the sympathetic and parasympathetic equilibrium. **(F)** Paracrine factors released by stem cells may effect electrophysiology of cardiomyocytes

Smit NW, Coronel R. ***Stem cells can form gap junctions with cardiac myocytes and exert pro-arrhythmic effects.*** ***Front Physiol 2014;5:419. doi: 10.3389/fphys.2014.00419.***

STEM CELLS

REGENERATIVE MEDICINE

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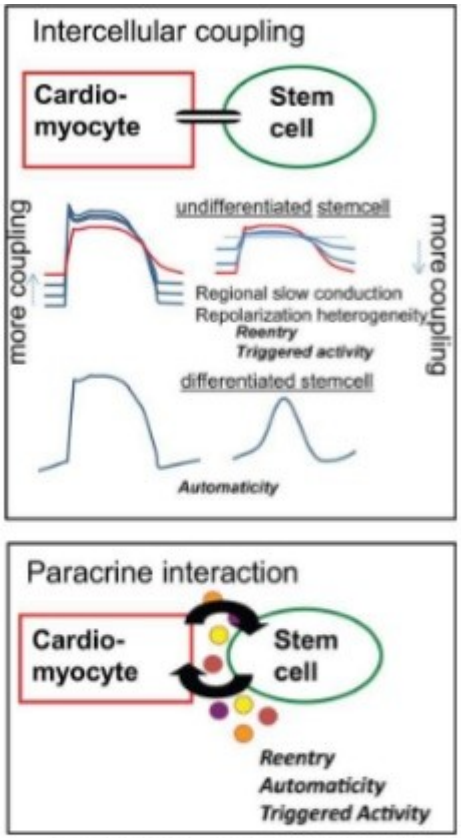
STEM CELLS 2016;34:34–43



Schematic representation of arrhythmogenic mechanisms caused by the interaction between stem cells and cardiomyocytes. Interaction can be by intercellular coupling (top panel) or by paracrine interaction (bottom panels). The more depolarized stem cells may induce depolarization in the coupled myocytes, depending on the degree of coupling and the relative size of the cells (blue and red action potentials). This may cause regional conduction slowing and regional repolarization prolongation, leading to reentry or triggered activity. If the stem cells are more differentiated and show spontaneous activity coupling to a myocyte may generate an automatic focus. Paracrine interaction may result from mutual interaction between the cell types (arrows) and may cause arrhythmogenic electrophysiological remodeling of the myocytes (leading to automaticity, triggered activity or reentry).

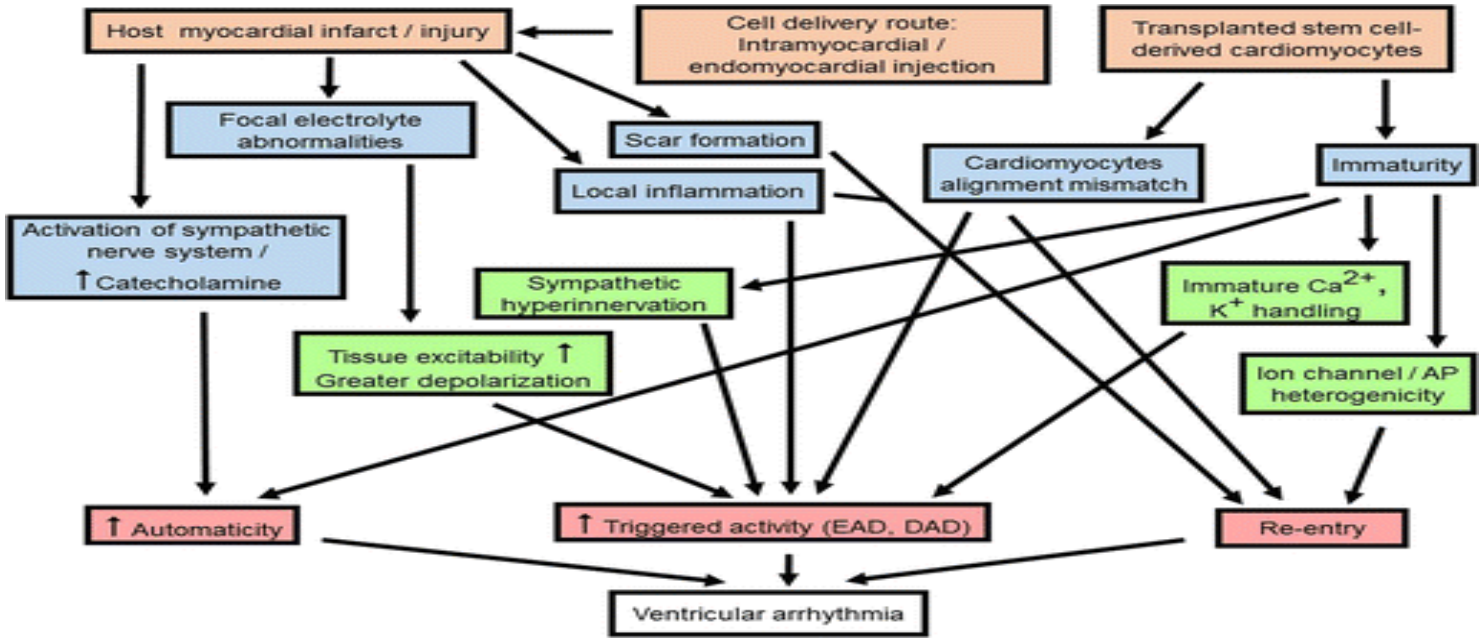
Post-MI hücre tedavisi sonrası gelişen ventriküler aritmilerin potansiyel mekanizmaları

Current Treatment Options in Cardiovascular Medicine
October 2016, 18:61

Arrhythmogenesis: a Roadblock to Cardiac Stem Cell Therapy

Authors Authors and affiliations

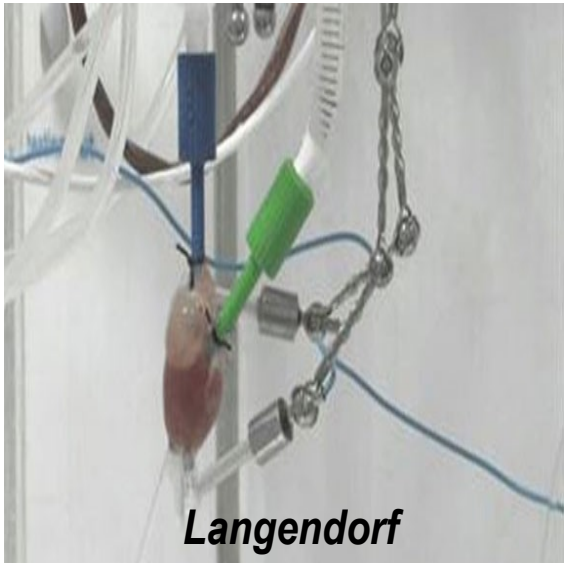
Yen-Wen Liu, Chi-Ting Su, Christopher Y. T. Yen, Li-Jen Lin, Patrick C. H. Hsieh



Artmış otomatisite
Re-entry
Tetikleyici aktivite

Ventriküler aritmi

Aritmi Modellerinin Geçerliliği



- Kalp hücreleri in vitro izole edildiğinde maruz kalınan çevre şartları fizyolojik değildir. Ör: Dolaşım yoktur.

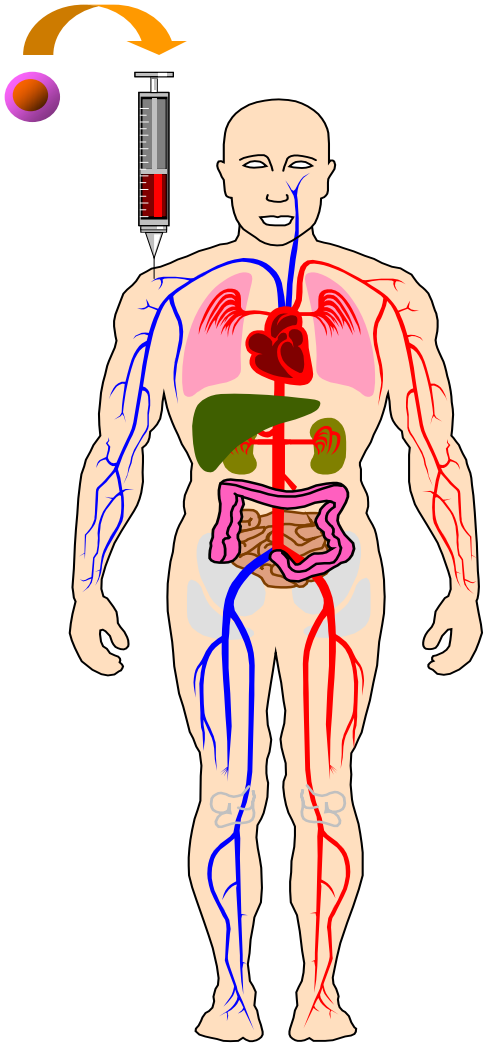
- İn situ kök hücre kaynaklı kardiyomiyositler matür hale getirilebilirse transplantasyon sonrası elektrofizyolojik özellikleri stabil hale gelebilir.

  (İnsan kardiyomiyositleri, normal gelişim süreçlerinde bir ile iki dekadda olgunlaşır. Kök hücre derive kardiyomiyositler ise in vitro olarak birkaç haftada oluşturulurlar ve en önemli karakteristikleri immatür olmalarıdır.

  Bu hücrelerin sarkoplazmik retikulumlarında, elektromekanik coupling için gerekli olan T tubuller eksiktir. Bu durum anormal hücre içi Ca^{+2} artışı ve sonuçta anormal depol/repol ve tetiklenmiş aktivite ile ilişkilidir.)

Antiaritmik Tedavide Kök Hücre Uygulamaları:

Bradiaritmiler



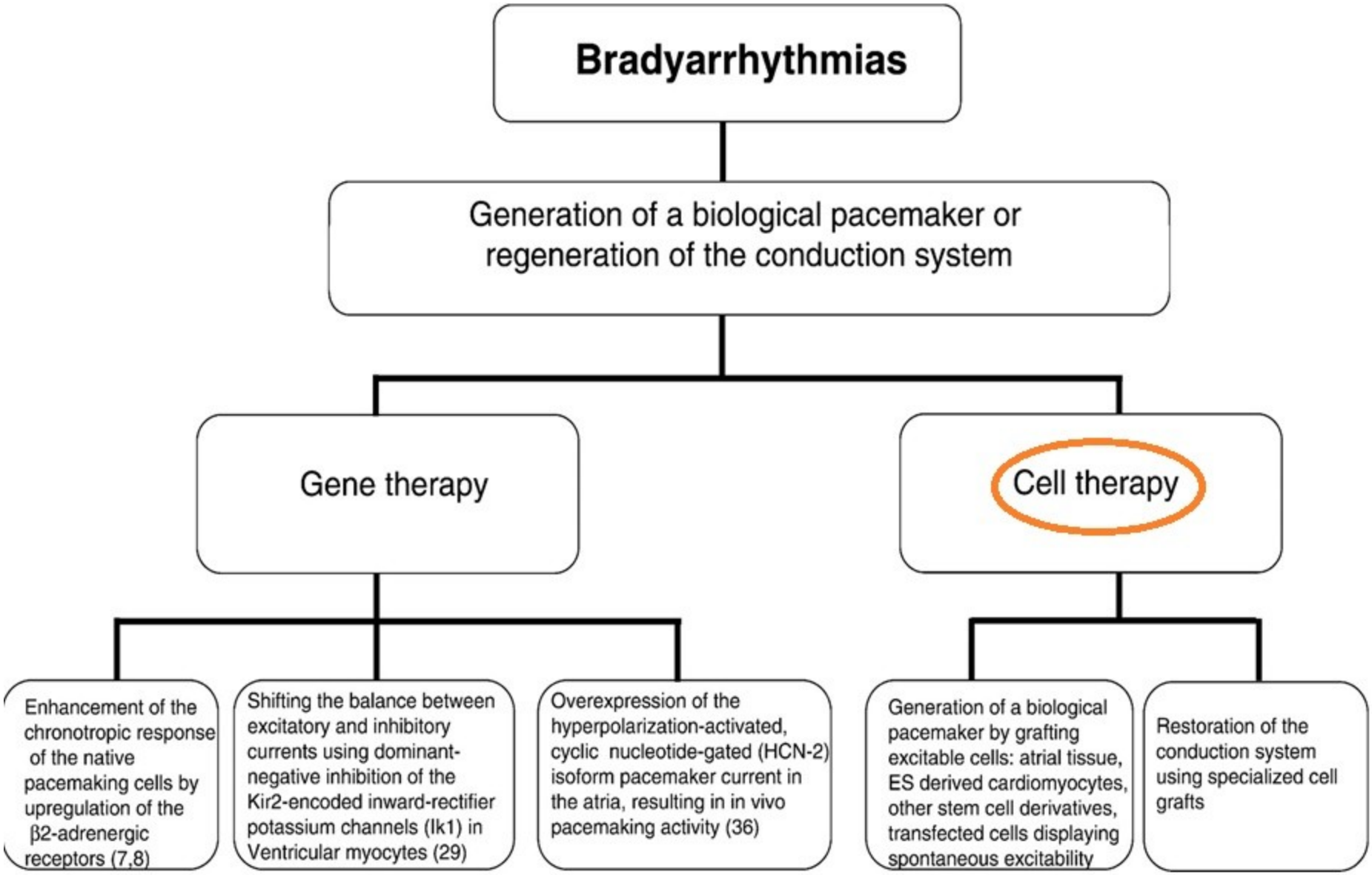
From Gene Therapy and Stem Cells to Clinical Electrophysiology

LIOR YANKELSON and LIOR GEPSTEIN

From the Sohnis Family Research Laboratory for the Regeneration of Functional Myocardium, Department of Biophysics and Physiology, the Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

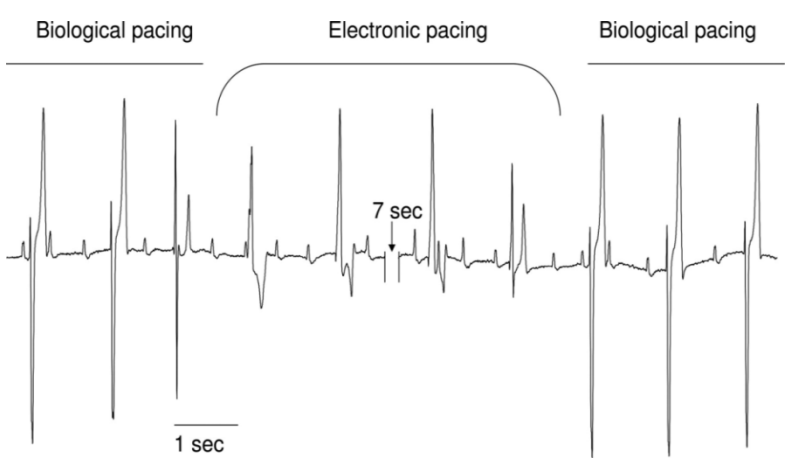
Gene therapy, cell therapy, and tissue engineering are emerging as novel experimental therapeutic paradigms for a variety of cardiovascular disorders. In the current report we will review the possible implications of these emerging technologies in the field of cardiac electrophysiology. Initially, the possible role of myocardial gene and cell therapies in creating a biological alternative to electronic pacemakers for the treatment of bradyarrhythmias will be discussed. This will be followed by a description of the possible applications of using similar strategies for the treatment of common tachyarrhythmias. Finally, the electrophysiological implications of cardiac stem cell therapy for heart failure, as well as the possible in vitro *applications of stem cell technology for electrophysiological studies and drug screening, will be discussed. While these emerging strategies provide a paradigm shift from conventional treatment modalities, this field is still at its infancy and several obstacles, discussed in this review, should be overcome before any clinical breakthroughs can be expected. (PACE 2006; 29:996–1005)*

Concept	Mechanism and Examples
Cell therapy for bradyarrhythmias	Biological pacemakers
1. Genetically engineering cells, *ex vivo*, to display pacemaking properties	Overexpression of the HCN-2 encoded pacemaker current in MSc
2. Directing the differentiation of stem cells to become cardiac pacemaking cells	*In vitro* differentiation of hESC
3. Creating conduction tissue	Using fibroblasts or hESC-CMs to bridge conduction
Cell therapy for tachyarrhythmias	
Modulating cardiac local or global electrophysiological properties	
Electrophysiological modification of cardiac tissue using genetically modified cells, transfected to express ionic channels	Grafting of fibroblasts expressing specific potassium channels (Kv1.3)
Decreasing the arrhythmogenic potential of cell therapy	
Improving coupling and conduction of grafted cells used in cell therapy	Overexpression of connexins in skeletal myoblasts
Indirect effects	
Treatment of the underlying pathology (ischemic heart disease, heart failure)	Transplantation of endothelial progenitor cells (CAD), stem cells and cardiomyocytes (heart failure)


Bradyarrhythmias
Generation of a biological pacemaker or regeneration of the conduction system
Gene therapy
Cell therapy
Enhancement of the chronotropic response of the native pacemaking cells by upregulation of the β2-adrenergic receptors (7,8)
Shifting the balance between excitatory and inhibitory currents using dominant-negative inhibition of the Kir2-encoded inward-rectifier potassium channels (Ik1) in Ventricular myocytes (29)
Overexpression of the hyperpolarization-activated, cyclic nucleotide-gated (HCN-2) isoform pacemaker current in the atria, resulting in in vivo pacemaking activity (36)
Generation of a biological pacemaker by grafting excitable cells: atrial tissue, ES derived cardiomyocytes, other stem cell derivatives, transfected cells displaying spontaneous excitability
Restoration of the conduction system using specialized cell grafts

Biyolojik Pacemaker Tedavisi

- Bradikardi tedavisi.
- KH kökenli kardiyomiyositler AP ve spontan atım aktivitesi oluşturabilirler.
- Stabil pacemaker potansiyeli gerekli.
- In vitro kardiyomiyojenik indükleme ile elde edilen kardiyomiyositlerin AP'leri değişebiliyor.



Miyoshi S, et al. Cardiac cell therapy and arrhythmias. Circ J 2007; Suppl A: A-45 - A-49

Biyolojik Pacemaker

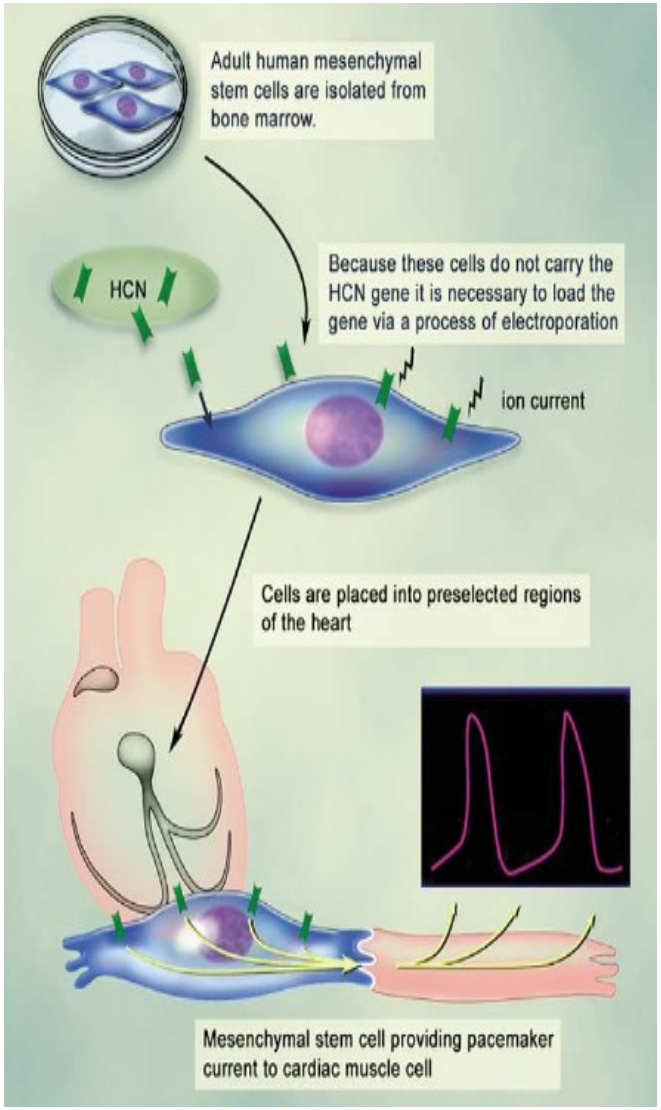
Biyolojik pacemaker fonksiyonları sağlamak için;

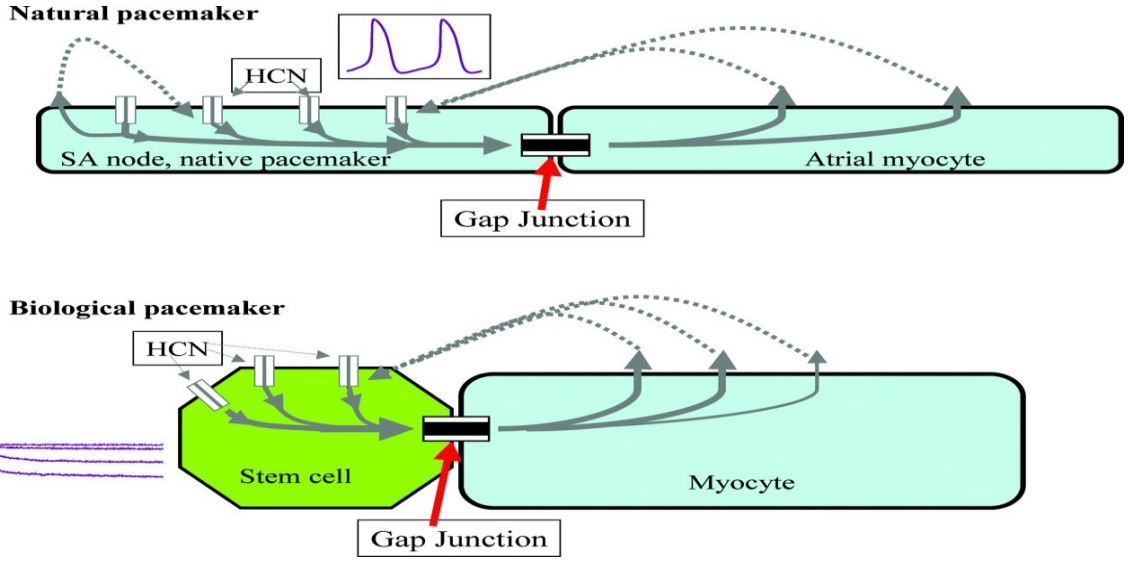
β2-adrenerjik reseptör upregülasyonu

IK1 (background K^+ akımı) downregülasyonu

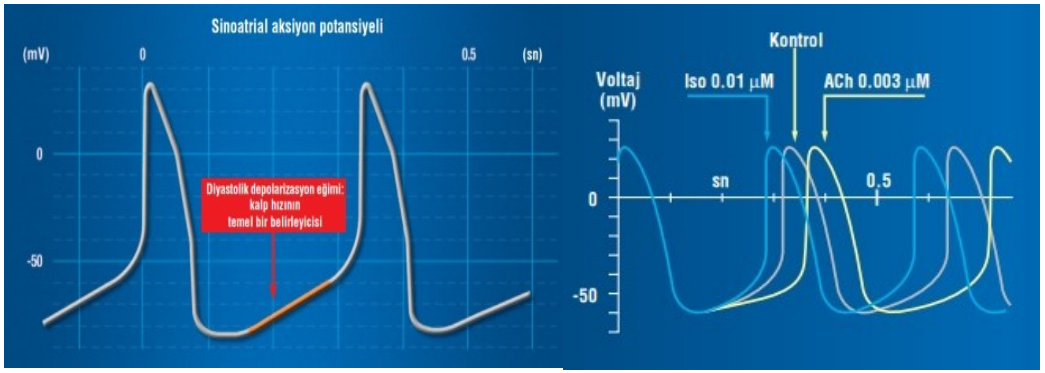
HCN2 gen (endojen kardiyak pacemaker If akımı) overekspresyonu

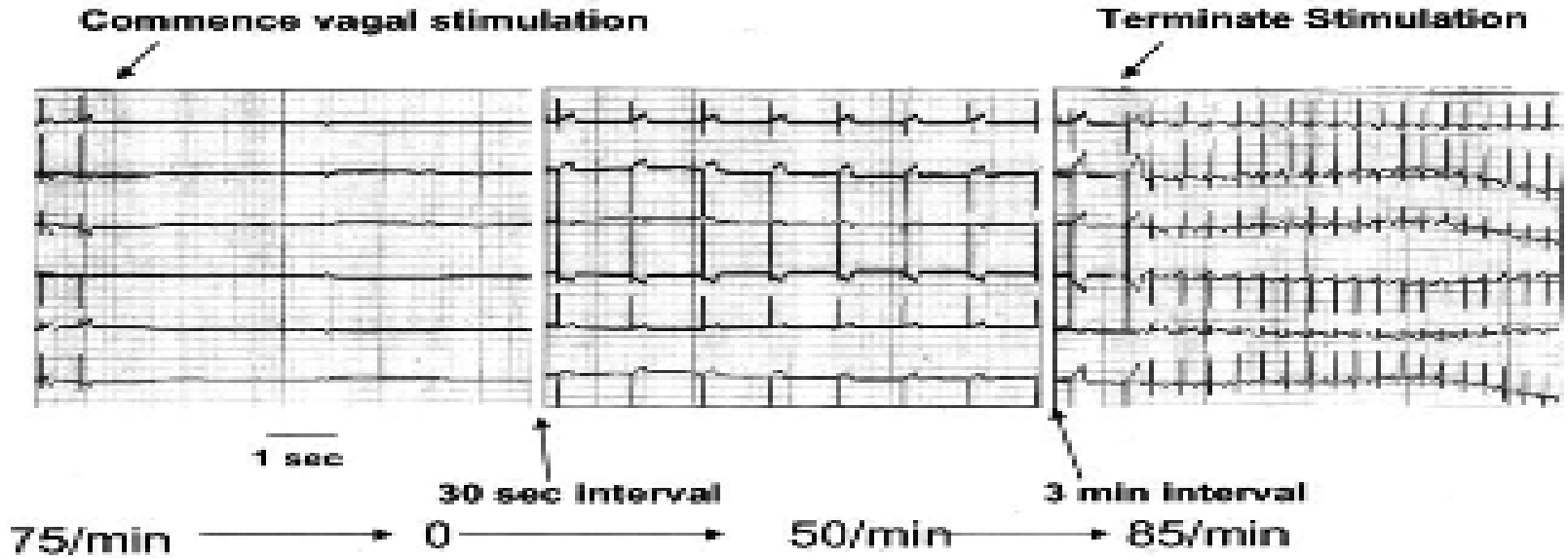
Biyolojik SAN – Mezenşimal Kök Hücre





Hyperpolarization-activated, Cyclic Nucleotide-gated (HCN) kanal (I_f current)





HCN-2 (*If* Kanalı)'e sahip mezenşimal KH köpeğin LV anterior duvarına implante edildikten 7 gün sonra vagal stimulasyon sonrası sinüzal arrest provake ediliyor.

Biyolojik pacemaker özelliğine sahip hücreler LV'den düzenli idioventriküler kaçış ritmi üretiyor.

Stimülasyon sonlandıktan sonra post vagal sinüs taşikardisi.

Potapova I, et al. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. Circ Res 2004;94:952-9.

Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 46, No. 10, 2005
ISSN 0735–1097/05/$30.00
doi:10.1016/j.jacc.2005.07.055

PRECLINICAL STUDY

Human Adult Bone Marrow Mesenchymal Stem Cells Repair Experimental Conduction Block in Rat Cardiomyocyte Cultures

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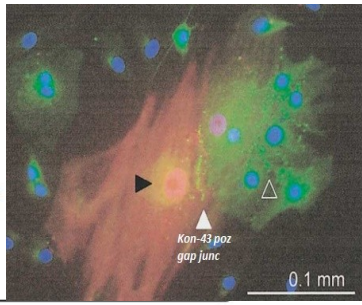
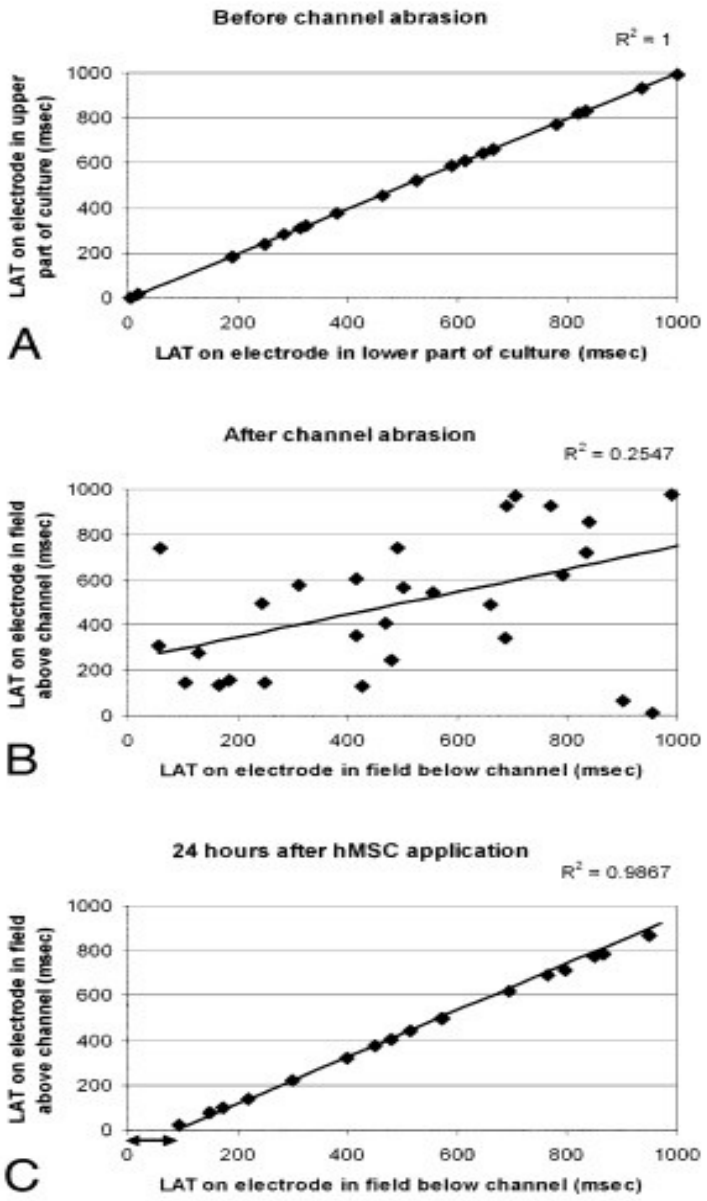
Leiden, the Netherlands



- ***İn vitro insan Kİ kökenli mezenşimal KH'ler, rat kardiyomiyosit kültürlerinde oluşturulan ileti bloğunu onarabilir.***
- KH-kardiyomiyosit ilişkili bölgelerde konneksin-43 izlenirken; kardiyomiyosit-miyoblast ya da kardiyomiyosit-fibroblast ilişkili bölgelerde izlenmemiştir.
- İnkübasyonu takiben, 48 saat içinde KH boyunca oluşan impulse iletiminin karakteristikleri yavaş akım, azalmış depol hızı ve düşük elektriksel aktivitedir.
- Yavaş akım proaritmojenik olabilse de; klinik çalışmalar bu yönde sonuçlanmamıştır.

Tse H-F, et al. Lancet 2003;361:47-9. ***Wollert KC, et al. Lancet 2004;364:141-8.***

AV Blok Tedavisi



- A. Kanal tahribinden önce kültürün alt ve üst bölgelerindeki lokal aktivasyon zamanları (LATs) arasındaki doğrusal ilişki senkron aktivasyon gösterir.
- B. Tahrip sonrası doğrusal ilişki kaybolmuş olup; senkron aktivasyon kaybolmuştur.
- C. KH uygulamasını takip eden 24 saatte senkronizasyon tekrar sağlanıyor. Fakat, kanal içindeki ileti gecikmesi nedeni ile alt bölgenin aktivasyonu ***80 ms gecikiyor***.

American Journal of Pathology, Vol. 169, No. 1, July 2006

DOI: 10.2353/ajpath.2006.051163

Cardiovascular, Pulmonary and Renal Pathology

Cardiac Conduction through Engineered Tissue

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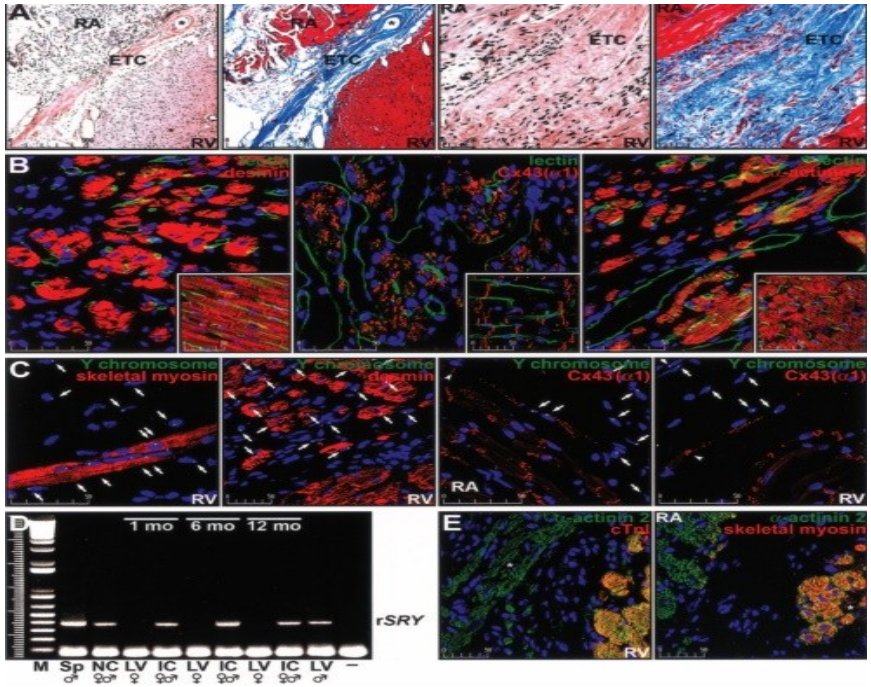
Peter E. Hammer,*† Kevin F. Kwaku,‡
Jennifer J. Marler,§ Ingeborg Friehs,*
Mara Jones,† Christine M. Rader,*† Nathalie Roy,*
Mau-Thek Eddy,† John K. Triedman,¶
Edward P. Walsh,¶ Francis X. McGowan, Jr.,†
Pedro J. del Nido,* and Douglas B. Cowan†

From the Departments of Cardiac Surgery, Anesthesiology,† Surgery,§ and Cardiology,¶ Children's Hospital Boston, and the Cardiovascular Division,‡ Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts*

Disruption of atrioventricular (AV) impulse propagation in the heart is a serious clinical problem in infants and children as well as in adults.[1–3] Congenital complete heart block or AV block because of ischemia, endocarditis, maternal systemic lupus erythematosus, or surgery is currently treated by implanting an artificial pacemaker device.[2,4] Although the efficacy of pacemakers as a palliative therapy cannot be disputed, and the range of indications requiring intervention with these devices continues to expand, their long-term performance remains primarily unsatisfactory, especially in pediatric patients.[3] Children have a substantially higher incidence of reop-



Doku mühendisliği desteği ile üretilecek olan graft (scaffold-based tissue gibi)'ler (ETCs) rejenere kardiyomiyositler aracılığı ile AV iletiyi restore edebilirler.

(Gap junction ve kas-spesifik proteinlerin mikroskobik görüntüleri)

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Regenerative Medicine

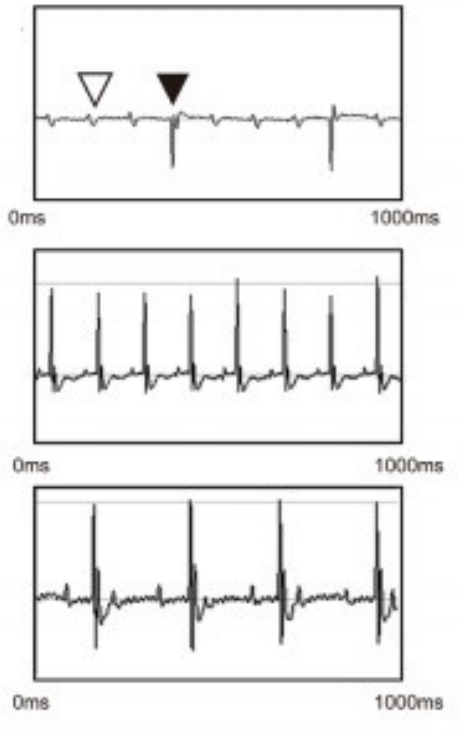
Regeneration of the Cardiac Conduction System by Adipose Tissue-Derived Stem Cells

Toshinao Takahashi, MD, PhD; Toshio Nagai, MD, PhD; Masato Kanda, MD, PhD; Mei-Lan Liu, MD, PhD; Naomichi Kondo, MD, PhD; Atsuhiko T Naito, MD, PhD; Takehiko Ogura, MD, PhD; Haruaki Nakaya, MD, PhD; Jong-Kook Lee, MD, PhD; Issei Komuro, MD, PhD; Yoshio Kobayashi, MD, PhD

Background: Adipose tissue is one of the sources of mesenchymal stem cells, which have the potential to differentiate into various types of cells, including myocytes. Whether brown adipose tissue (BAT)-derived cells might differentiate into the cardiac pacemaking-conducting cells, and have the potential to regenerate the cardiac conduction system (CCS), is investigated in this study.

Methods and Results: BAT was isolated from the interscapular area of mice and enzymatically digested before culture. Round or fusiform cells showed spontaneous beating at 4–7 days after culturing of BAT-derived cells. Reverse transcriptase-polymerase chain reaction analysis and immunocytochemical analysis revealed that BAT-derived cells expressed several cardiomyocytes, the CCS and pacemaker (PM) cell marker genes and proteins. Patch-clamp techniques revealed that spontaneous electrical activity and the shape of the action potential showed properties of cardiac PM cells. Next, a complete atrioventricular (AV) block was created in mice and green fluorescent protein-positive (GFP (+)) BAT-derived cells were injected intramyocardially around the AV node. At 1 week after transplantation, 50% of BAT-derived cells injected mice showed a sinus rhythm or a 2:1 AV block. Immunohistochemical analysis revealed that injected GFP (+) cells were engrafted and some GFP (+) cells co-expressed several cardiac PM cell marker proteins.

Conclusions: BAT-derived cells differentiate into the CCS and PM-like cells in vitro and in vivo, and may become a useful cell source for arrhythmia therapy. (*Circ J* 2015; **79:** 2703–2712)



- ***Adipoz doku*** önemli bir mezenşimal KH kaynağıdır.
- Kahverengi adipoz doku kaynaklı KH'ler kardiyak pacemaker hücrelerine dönüşüp ileti sistemini restore edebilirler.
- Farelerde komplet AV blok oluşturuldu kahverengi adipoz doku kaynaklı KH'ler intramiyokardiyal olarak AVN bölgesine enjekte edildi.
- 1 hafta sonra enjekte edilen hücrelerin %50'si 2:1 blok veya NSR gösterdi.

Niçin Biyolojik Pacemaker?

- Otonomik kontrolü manipüle edebilme,

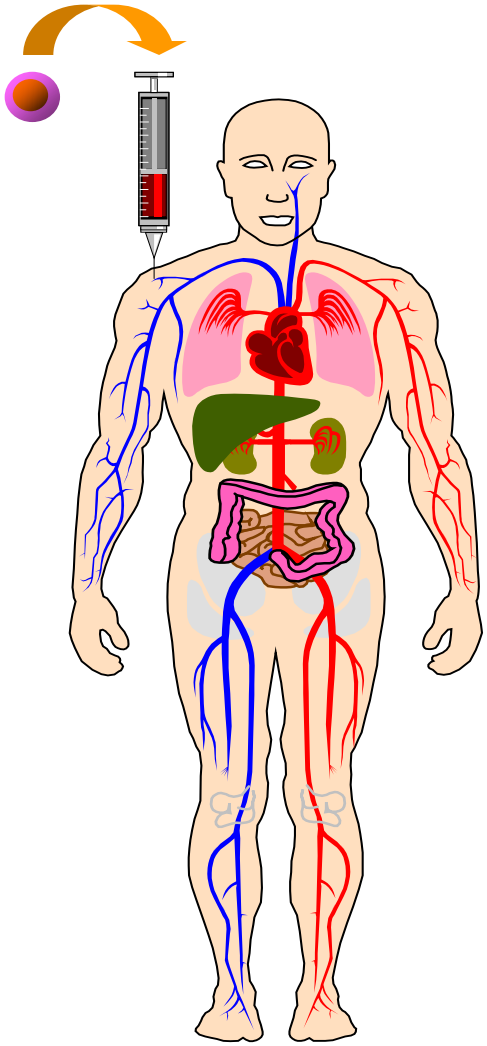
- Pacemaker hücre varlığını oluşturabilmek için iyon kanal sayısı, yapısı ve/veya fonksiyonuna müdahale edebilme,

ya da

sıfırdan SAN veya AVN oluşturmak

Antiaritmik Tedavide Kök Hücre Uygulamaları:

Taşiaritmiler



Taşiaritmiler

- Biyolojik pacemaker potansiyeline sahip hücrelerin nakli ile kardiyomiyositlerin elektrofizyolojik özellikleri modüle edilebilir.
- İyon kanalları, gap junction proteinlerinin düzenlenmesi ile hız ve ritim kontrolü sağlanabilir.
- Özellikle AF ve VT'lerde kullanılabilir.

Sasano T, et al. Molecular ablation of ventriculartachycardiaaftermyocardialinfarction.NatMed 2006;12:1256-8.

Kikuchi K, et al. Targeted modification of atrial electrophysiology by homogeneous transmural atrial gene transfer. Circulation 2005;111:264-70.

Circulation

ARRHYTHMIA/ELECTROPHYSIOLOGY

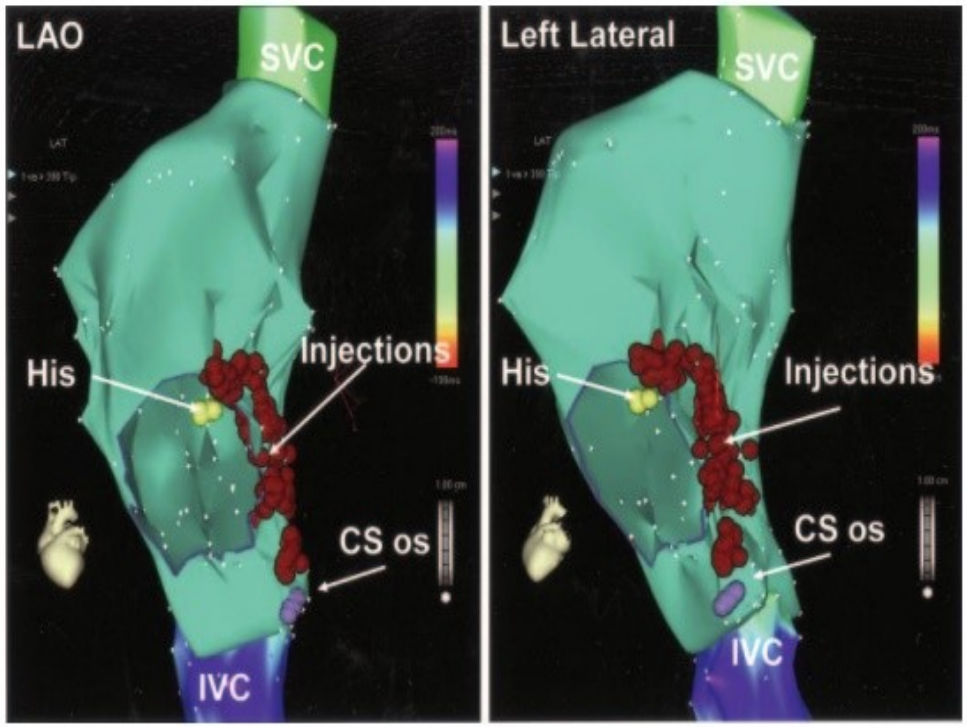
Impact of Transforming Growth Factor-β1 on Atrioventricular Node Conduction Modification by Injected Autologous Fibroblasts in the Canine Heart

T. Jared Bunch, Srijoy Mahapatra, G. Keith Bruce, Susan B. Johnson, Dylan V. Miller, Benjamin D. Horne, Xiao-Li Wang, Hon-Chi Lee, Noel M. Caplice, Douglas L. Packer

Download PDF

DOI https://doi.org/10.1161/CIRCULATIONAHA.105.570796
Circulation. 2006;113:2485-2494
Originally published May 30, 2006

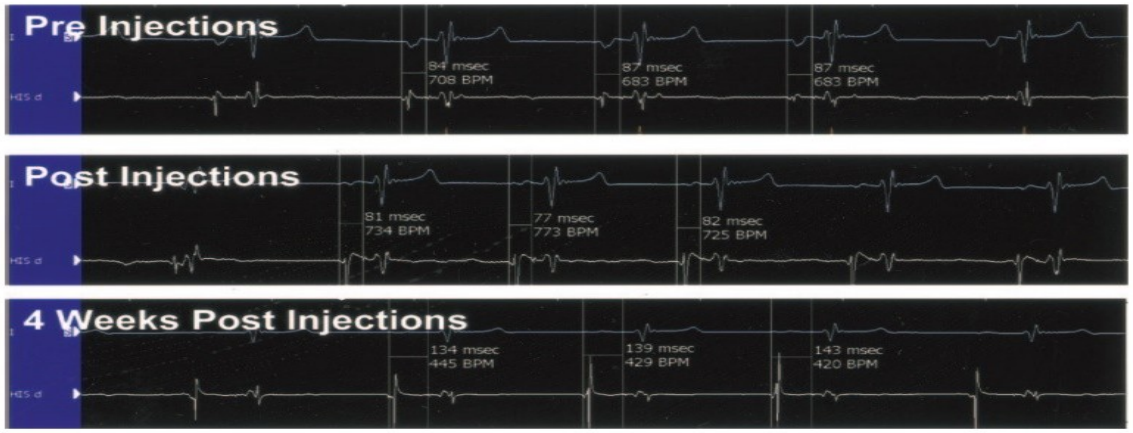
AF tedavisinde AVN ablasyonu geri dönüşümsüz olup kalıcı pacemaker implantasyonu gerektirir.



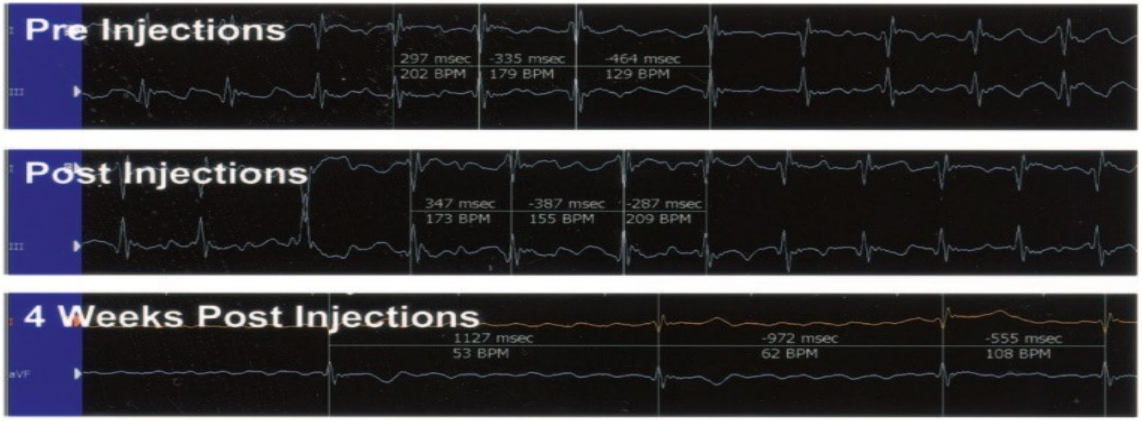
NOGA enjeksiyon kateteri

Electroanatomic mapping guidance of therapy injections. Left anterior oblique (LAO) and left lateral views are displayed. The His and CS ostium positions are marked. Injections are indicated by lesion markers.

Mongrel cinsi köpeklerden cilt biyopsisi ile elde edilen ve TGF-β1 ile stimüle edilen fibroblastlar elektroanatomik mapping kılavuzluğunda 8F NOGA kateter ile hızlı/yavaş yol (AVN) boyunca enjekte edildi.



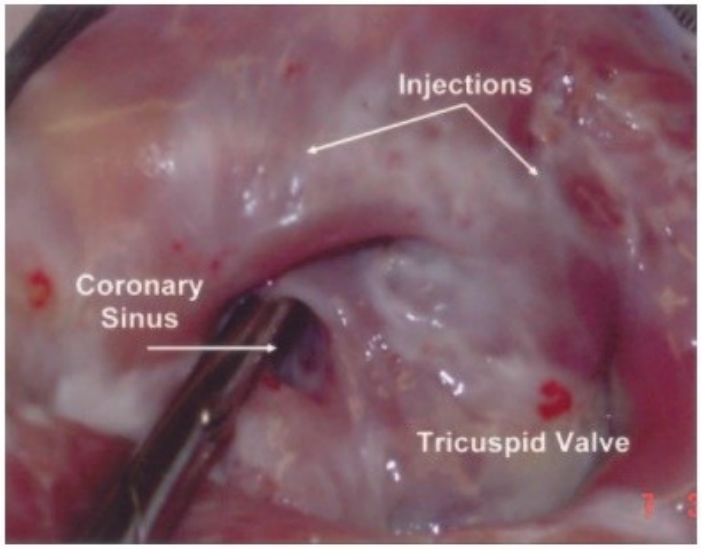
An example of AH interval prolongation after fibroblast+TGF-β1 combination therapy. Individual electrograms represent the AH interval before injections, immediately afterward, and 4 weeks later.



Example of RR interval prolongation during pacing-induced AF after fibroblast+TGF-β1 combination therapy. Individual electrograms represent the AH interval before injections, immediately afterward, and 4 weeks later.

Fibroblast enjekte edilen grupta (*fokal skar ile elektriksel ve mekanik etki*) AH süresinde anlamlı uzama saptandı.

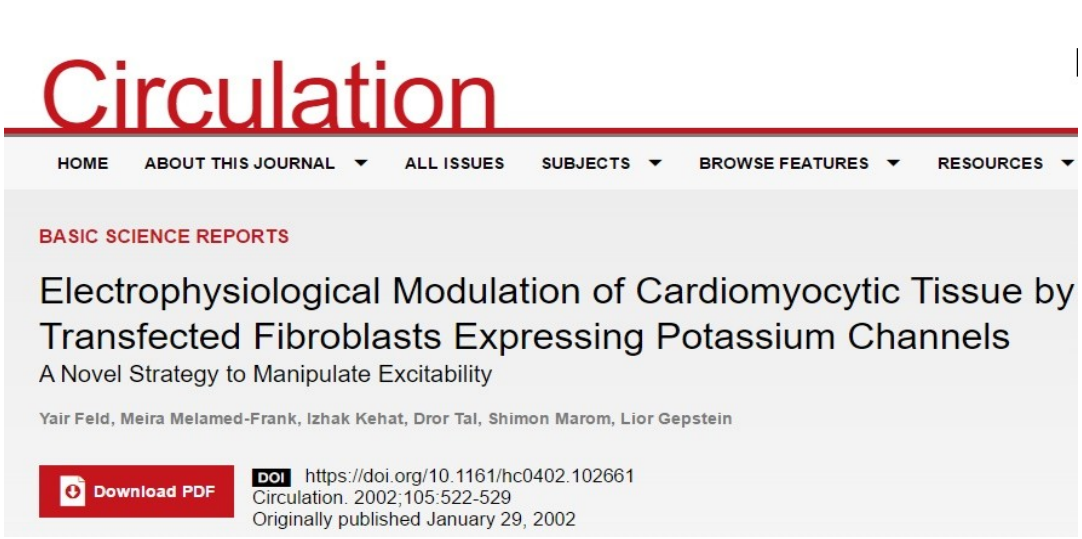
AV blok olmaksızın AF'de hız kontrolü sağlandı.

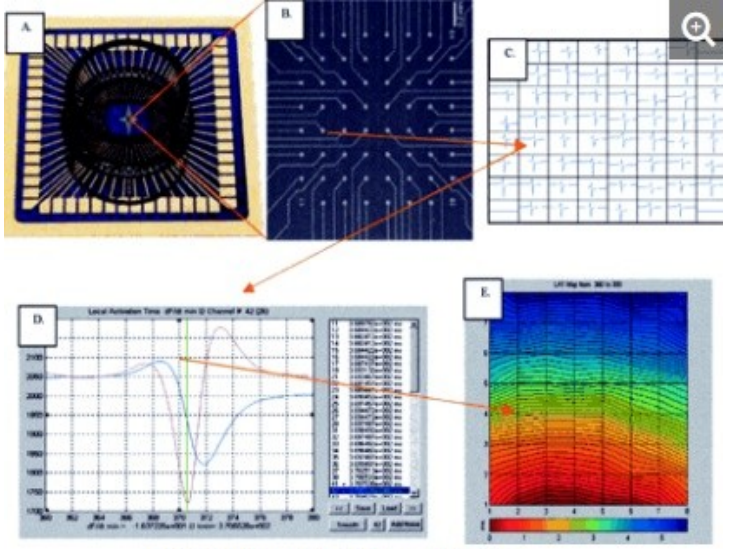


Pathology of the autologous fibroblast injection lesions. Gross appearance of the peri-AV nodal RA 4 weeks after injection (arrows indicate injection injury; arrowheads denote the tricuspid valve; a hemostat is within the CS ostium).

Fokal verilmesine rağmen RF ile uzak yapılarda olabilecek istenmeyen hasarlara karşın; fibroblast verilmesini takiben bu olumsuzluklar izlenmez.

İlgi çekici bir tedavi seçeneği olabilir.





Multielektrod haritalama tekniği

Voltaj duyarlı Kv1.3 K^+ kanalları ile transfekte edilmiş fibroblastlar, var olan kardiyomiyosit kültürleri ile yapısal ve fonksiyonel olarak birleşebilir.

Transplante hücreler, hibrid kültürlerinin ileti özelliklerinin değiştirebilir.

Spesifik Kv1.3 kanal blokeri (Charybdotoxin) verilmesi ile elektrofizyolojik etkiler geri döndürülebilir.

Lokal refrakter periyodda uzama ve lokal otomatisitede azalma gibi etkiler antiaritmik tedavide kullanılabilir.

Nat Biotechnol. 2007 Sep;25(9):1015-24. Epub 2007 Aug 26.

Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts.

Laflamme MA[1], Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE.

İnsan embriyonik KH'lerinden elde edilen kardiyomiyositlerin Mİ geçiren farelere enjeksiyonu sonrası, infarktlı bölgelerin iletimi güçlenmiş ve ventriküler aritmi sıklığı azalmıştır.

Post-MI hücre tedavisi sonrası gelişen ventriküler aritmiler- Tedavi seçenekleri

- Elektrolit dengesizliğinin düzeltilmesi, asit-baz dengesinin sağlanması, miyokard perfüzyonunun optimizasyonu, KY gibi post-Mİ komplikasyonların tedavisi, kontrendikasyon yoksa β bloker verilmesi, gereğinde ICD takılması (*ACC/AHA*).
- Enjeksiyon yolunu belirlemek,
- İn-vitro matür hücre oluşumunu sağlamak,
- Cell-seeded patches ya da scaffold-free cell sheets gibi ***doku mühendisliği***ndeki gelişmeler graft ve alıcı miyokardiyumu arasındaki hücrelerarası bağlantıları geliştirebilir; bu durum ventriküler aritmi oluşma riskini azaltır.

Lin YD, et al. A nanopatterned cell-seeded cardiac patch prevents electro-uncoupling and improves the therapeutic efficacy of cardiac repair. Biomater Sci 2014;2:567-80.

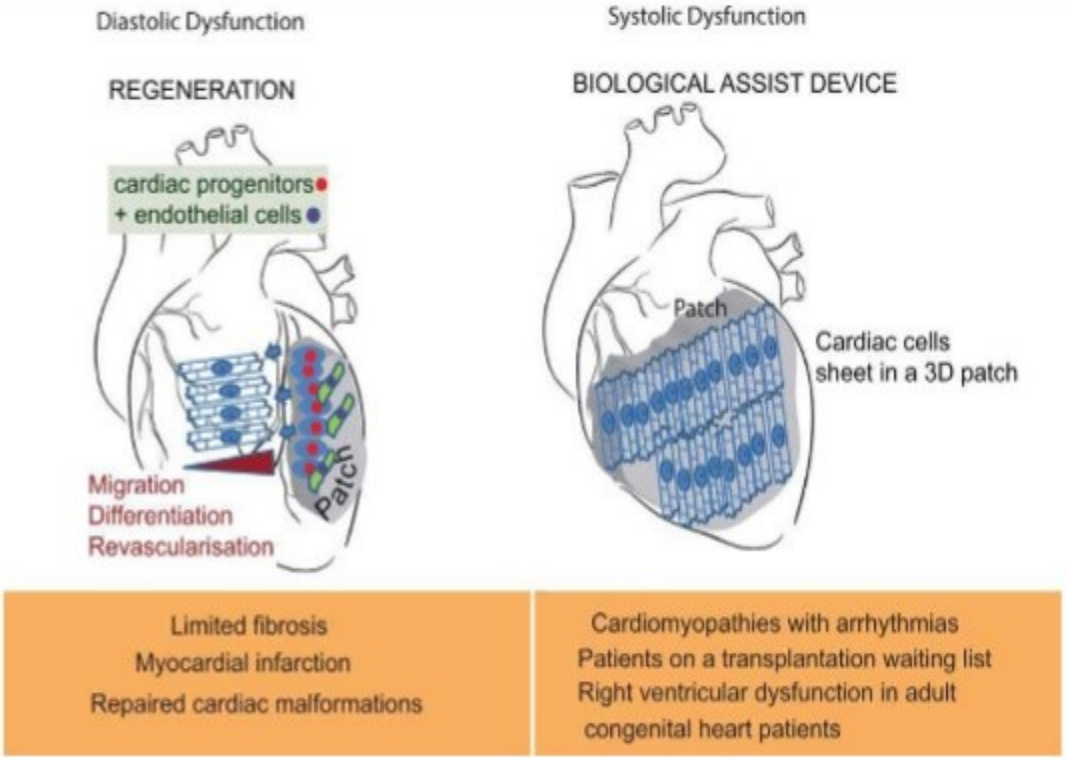
STEM CELLS

REGENERATIVE MEDICINE

Concise Review: Pluripotent Stem Cell-Derived Cardiac Cells, A Promising Cell Source for Therapy of Heart Failure: Where Do We Stand?

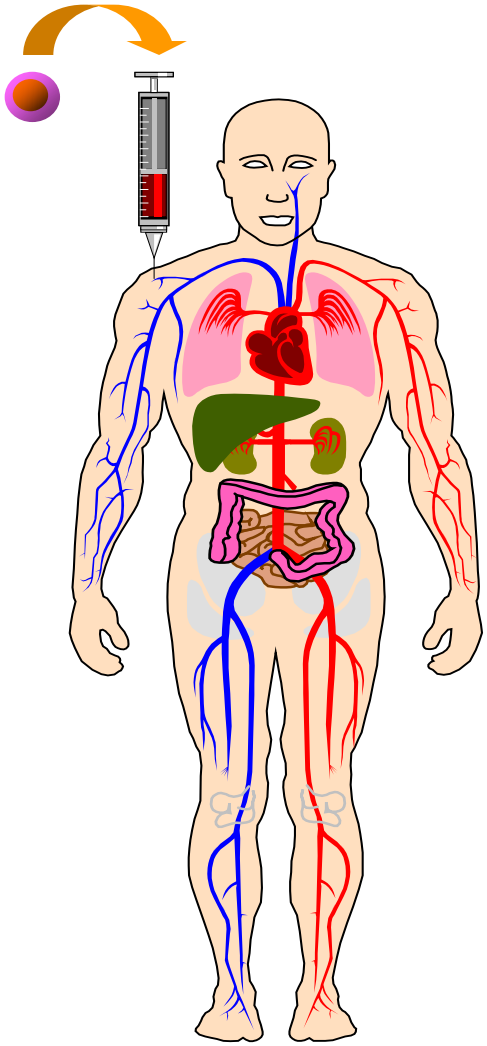
ELODIE GOUADON,[a] THOMAS MOORE-MORRIS,[b] NICOLINE W. SMIT,[c] LUCIENNE CHATENOUD,[d] RUBEN CORONEL,[c] SIAN E. HARDING,[e] PHILIPPE JOURDON,[a] VIRGINIE LAMBERT,[a] CATHERINE RUCKER-MARTIN,[a] MICHEL PUCÉAT[b]

STEM CELLS 2016;34:34–43



Cell based therapeutic interventions to restore ventricular function under different pathological conditions. Diastolic dysfunction requires both myocardial regeneration and vascularization by adding both new endothelial cells and cardiac progenitor cells differentiating toward cardiomyocytes that can repopulate the myocardium. Systolic dysfunction requires more an assisting biological device such a cardiac cell sheet (engineered from pluripotent stem cells) wrapping the whole heart.

Antiaritmik Tedavide Kök Hücre Uygulamaları: *CRT*



Kardiak Resenkronizasyon Tedavisi (CRT)

- KY'li, geniş QRS'li ve LBBB'lu hastalarda elektriksel ve mekanik dissenkronizasyon görülür.
- Cihaz tedavisinin olumlu etkileri kanıtlanmıştır.
- Günümüzde miyositler ile kompleks sinyal yolaklarını aktive ederek CR'u yeniden sağlamak; bu sayede KY ve düşük EF üzerine olumlu katkılarda bulunmak ve aritmileri önlemek amaçlanmaktadır.

VIA MEDICA

ORIGINAL ARTICLE

Cardiology Journal
2013, Vol. 20, No. 3, pp. 304–309
DOI: 10.5603/CJ.2013.0076

ISSN 1897–5593

Acute effects of cardiac resynchronization therapy on arterial distensibility and serum norepinephrine levels in advanced heart failure

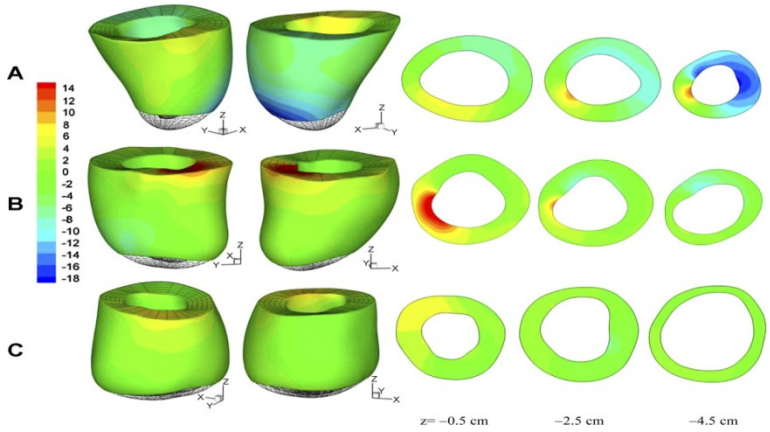
Mustafa Yildiz[1], Hakan Hasdemir[2], Ceyhan Turkkan[2], Mehmet Ali Astarcioglu[3], Ahmet Taha Alper[2], Alparslan Sahin[4], Mehmet Ozkan[3]

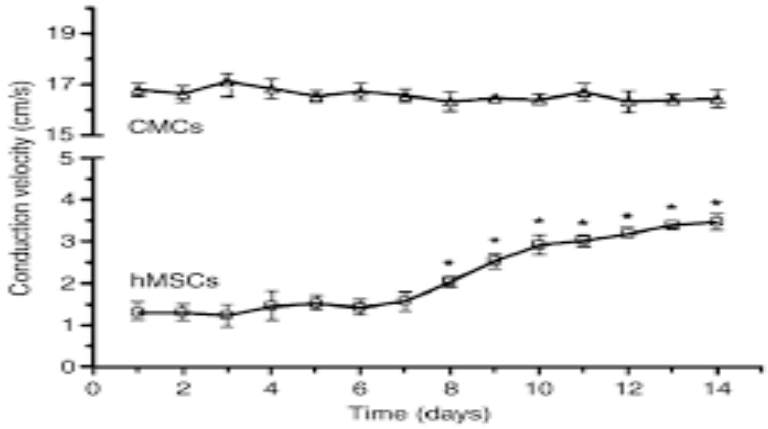
CRT

Kİ kökenli KH kullanılarak yapılan resenkronizasyon tedavisi LV kontraksiyon paternine katkı ile semptomatik düzelme sağlar.

Kİ kökenli KH tedavisi dissenkronizasyonda azalma, LVEF'nda anlamlı artış yapmıştır.

Kİ kökenli KH neovaskülarizasyon, konak kardiyomiyositler ile olan elektriksel bağlantıların düzenlenmesi ile intraventriküler iletide düzelme sağlamaktadır.



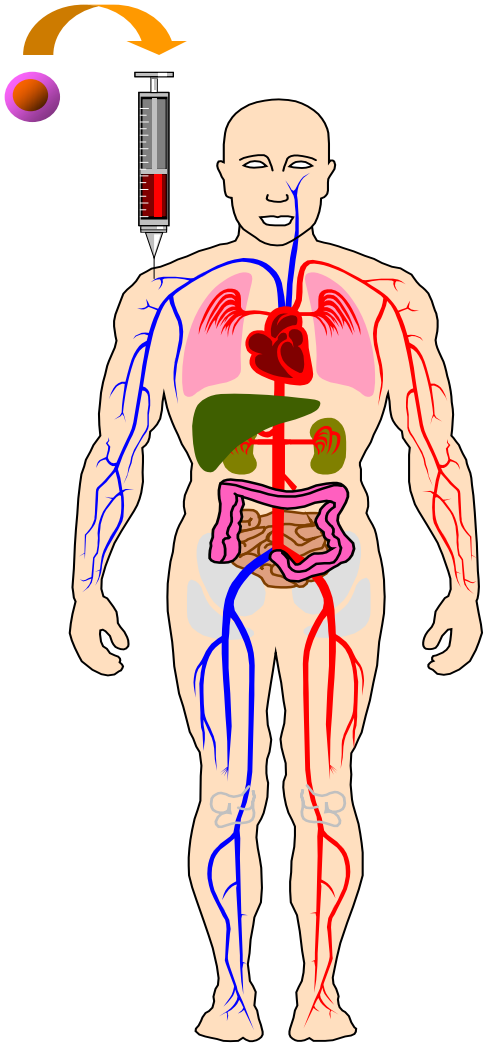


van Ramshorst J, et al. Effect of intramyocardial bone marrow cell injection on left ventricular dyssynchrony and global strain. Heart 2009;95:119-24.

Chang SA, et al. Restoration of left ventricular synchronous contraction after acute myocardial infarction by stem cell therapy: new insights into the therapeutic implication of stem cell therapy for acute myocardial infarction. Heart. 2008;94:995-1001.

Antiaritmik Tedavide Kök Hücre Uygulamaları:

Kalıtsal Aritmiler



Kalıtsal Aritmilerde Kök Hücre Tedavisi

- Herediter uzun QT, Katekolaminerjik polimorfik VT,….
- Kalıtsal aritmilerin günümüzdeki tedavi stratejisi: β bloker, ICD implantasyonu, sol ventrikül sempatik denervasyonu,…
- Bu yaklaşımlar ampirik tedaviyi oluşturuyor. Ani kardiyak ölümü önlemede %100 etkin değil!
- Hücre bazlı tedaviler, pluripotent kök hücreden kardiyomiyosit üretimi ve transplantasyonu önemli yaklaşımlardan biridir.
- Çalışmaların çoğu küçük hayvanlarla yapılmıştır.
- Yapılan in vitro çalışmalar ve hayvan deneyleri, klinik pratikte kök hücre tedavisini henüz pek mümkün kılmamaktadır.

Circ J 2017; 81: 12–21
doi:10.1253/circj.CJ-16-1113

REVIEW

Modeling Inherited Arrhythmia Disorders Using Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Vassilios J. Bezzerides, MD, PhD; Donghui Zhang, PhD; William T. Pu, MD

Inherited arrhythmia disorders (IADs) are a group of potentially lethal diseases that remain diagnostic and management challenges. Although the genetic basis for many of these disorders is well known, the pathogenicity of individual mutations and the resulting clinical outcomes are difficult to predict. Treatment options remain imperfect, and optimizing therapy for individual patients can be difficult. Recent advances in the derivation of induced pluripotent stem cells (iPSCs) from patients and creation of genetically engineered human models using CRISPR/Cas9 has the potential to dramatically advance translational arrhythmia research. In this review, we discuss the current state of modeling IADs using human iPSC-derived cardiomyocytes. We also discuss current limitations and areas for further study.

Genetics of Inherited Arrhythmia Disorders

Disorder / Causative genes	Cellular phenotype	Clinical phenotype
LQTS		
KCNQ1 (LQT1), KCNE1	Decreased I_{Ks}	TdP
KCNH2 (LQT2), KCNE2	Decreased I_{Kr}	TdP
SCN5A (LQT3), SCN4B, SNTA1	Increased I_{Na}	TdP, bradycardia
ANK2	Decreased Na/K-ATP activity	Bradycardia, conduction block, TdP
KCNJ2 (Andersen-Tawil syndrome)	Decreased I_{K1}	Facial anomalies, periodic paralysis, stress-induced VT
CACNA1C (Timothy syndrome)	Decreased I_{Ca}	TdP, autism, syndactyly
CAV3	Decreased I_{Kr}	TdP
CALM1, CALM2	Altered calcium handling	VT
CPVT		
RYR2	Increased calcium leak, altered calcium handling	Stress and exercise-induced VT/VF
CASQ2	Decreased SR calcium buffering	Stress and exercise-induced VT/VF
CALM1	Abnormal Ca^{2+} signaling	Stress and exercise-induced VT/VF/long QT
Triadin	Altered release of Ca^{2+}	Stress and exercise-induced VT/VF
BrS		
SCN5A, SCN10A, SCN1B, SCN2B, SCN3B, GPD1L, MOG1, SLMAP, PKP2, HEY2	Decreased I_{Na+}	ECG changes, VT/VF at rest or with fever
CACNA1C, CACNB2, CACNA2D1	Decreased I_{Ca2+}	VT/VF at rest
HCN4, KCNE3, KCNE5, KCND3, ABCC9, KCNJ8, KCNH2, PKP2	Increased I_{K+}; I_{to}	VT/VF
ACM		
PKP2, DSG2, DSC2, DSP, JUP	Decreased I_{Na} and gap junctions; apoptosis	Fibrosis, cardiac dysfunction, VT

ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; LQTS, long QT syndrome; Tdp, torsades de pointes; SR, sarcoplasmic reticulum; VF, ventricular fibrillation; VT, ventricular tachycardia.

Kök Hücre Tedavisi - Kısıtlılıklar

- Yüksek sayıda hücre üretmek
- Pro-aritmik etkiler

  (Aritmilerin büyük çoğunlu re-entry mekanizması ile ortaya çıkıyor ve izole edilen hücre grubunda bunu çalışmak mümkün olmuyor)
- Maliyet ve güvenilirlik
- Teratom formasyon riski (?)
- Eksik veya kemik-kıkırdak gibi uygunsuz hücrelere farklılaşmalar
- Genetik ve epigenetik anomaliler
- Transplantasyon sonrası immünolojik problemler

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Pluripotent stem cells

Focus issue: **March 2016** Volume 17, No 3

Pluripotent stem cells progressing to the clinic

Maymun MI modelinde 1 milyar embriyonik KH kaynaklı kardiyomiyosit intramiyokardiyal verildiğinde; remuskülarizasyon ve non-fatal ventriküler aritmiler izlendi.

CABG op'na giden bir hastada, infarkt sahasına aynı zamanda embriyonik KH kaynaklı kardiyomiyosit progenitörleri verildi. 3 ay sonra kardiyak semptomlarda düzelme izlenirken; aritmi görülmedi.

Heart disease. Despite concerns and disappointments over cell therapeutic studies for the repair of the serious cardiac muscle damage that results from heart attacks[61,62], recent studies have shown that cardiac muscle derivatives of human ES cells can halt the deterioration of cardiac function and improve experimentally induced diminished heart function in rodent and monkey models[63,64]. Moreover, intramyocardial delivery of 1 billion human ES cell-derived cardiomyocytes in a monkey myocardial infarct model has shown extensive remuscularization of myocardial infarcts; however, non-fatal ventricular arrhythmias were observed[64].

ES cell-derived cardiomyocytes have been shown to perform better than mononucleated blood cells for repair of myocardial infarct[63]. A single patient who had a coronary bypass operation, at the same time was grafted in the infarct area with human ES cell-derived cardiomyocyte progenitors expressing the cardiac transcription factor insulin gene enhancer ISL1 and stage-specific embryonic antigen 1 (SSEA1), which are cell markers for cardiac progenitors. The cardiac progenitors were embedded in fibrin to enable a patch to be grafted to the damaged heart, enabling integration of the grafted cells into heart tissue[65]. No adverse events were associated with the graft, and the symptoms of heart disease improved after 3 months, although this may have been owing to revascularization resulting from the bypass surgery. Notably, contractility was observed in the previously akinetic heart patch area, and no arrhythmias were evident[65,66].

Box 1 | **Tissue stem cells emerge as a new paradigm in human medicine**

Bone marrow transplants, using bone marrow engrafting haematopoietic stem cells (HSCs), are well-established therapies for the treatment of blood diseases and cancers.

Bone marrow stromal cells, often termed mesenchymal stem cells (MSCs), are being studied in hundreds of clinical trials for a wide array of disease conditions, with some demonstrated clinical benefits for immune rejection, as in paediatric graft-versus-host disease (GVHD), bone repair and joint or lower back pain. Stromal cells from other tissues, such as placenta, umbilical cord and adipose tissue, are also in clinical trials for many of the same applications, with variable outcomes so far. Other applications of MSCs, such as therapy for myocardial infarct, diabetes, pulmonary diseases, neurological diseases, cartilage repair and liver disease, are less promising at the present time[1].

Neural stem cells from adult and fetal sources are being studied for many neurodegenerative disorders and diseases of the eye[1,3]. The neural stem cells are glial or neuron progenitors, and some have been immortalized and some engineered to overexpress neural growth factors such as glial derived neural growth factor (GDNF). Depending on the disorder, the neural stem cell type would be expected to, for example, insulate the myelin sheaths of neurons damaged in spinal cord injury, protect motor neurons in amyotrophic lateral sclerosis (ALS) or provide dopamine-secreting A9 dopaminergic neurons in Parkinson disease.

Limbal stem cells of the cornea have been approved for autologous therapies, in which the healthy limbal cells can be recovered from the patient's own eyes. The use of these cells as allogeneic transplants that involve donor cells (live or cadaveric sources) are also progressing well in clinical trials for the restoration of sight in cases of corneal burns[1].

Gene therapy using the research participant's own haematopoietic stem cells that are DNA-edited to replace a mutated gene with a normal copy is being used in clinical trials to successfully cure single-gene diseases such as thalassaemia, sickle cell disease, adrenoleukodystrophy and severe combined immunodeficiency[76,77].

Therapies that disrupt the function of crucial blood cell co-receptors for HIV infections, such as by mutating the chemokine receptor 5 gene (*CCR5*), or inhibiting its function using short inhibitory RNA technology, are being trialled as potential cures for AIDS[78,79]. The *CCR5* gene encodes a co-receptor that enables HIV to enter T cells and macrophages.

Probably the most advanced clinical trials for cell therapies at present are those using chimeric antigen receptor technologies (CAR-T) to increase the effectiveness of killer T cell destruction of acute lymphoblastic leukaemia (ALL), multiple myeloma and glioblastoma[80,81,82]. The research participant's own T cells are recovered and transfected with a tumour-recognition molecule, such as an antibody CD19 antigen, together with co-stimulatory and activating domains that mediate T cell lysis of tumour cells. The engineered CAR-T CD19 T cells are expanded *ex vivo* and re-infused back into the research participant. For ALL, clinical success has been reported to be as high as ~90% complete remission after 6 months.

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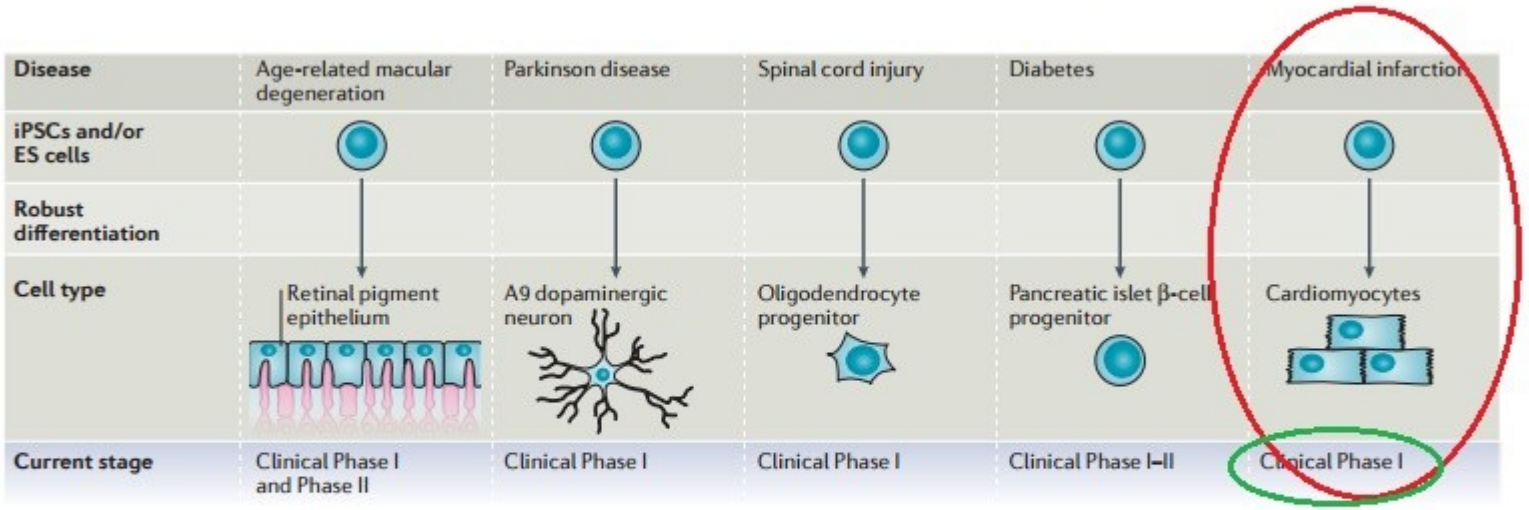
PLURIPOTENT STEM CELLS



Faz 0 – Preklinik Çalışmalar;

Geliştirilen ilacın deney hayvanlarında ya da insanlarda mikrodozlar halinde uygulanarak etkene verilen cevap araştırılır.

Faz 1 Çalışmalar;

İlacın farmakokinetik özellikleri, toksisitesi, biyoyararlanımı,farmakolojik etkileri az sayıda sağlıklı gönüllüde araştırılır. Bu fazın ana amacı güvenliliktir.

Faz 2 Çalışmalar;

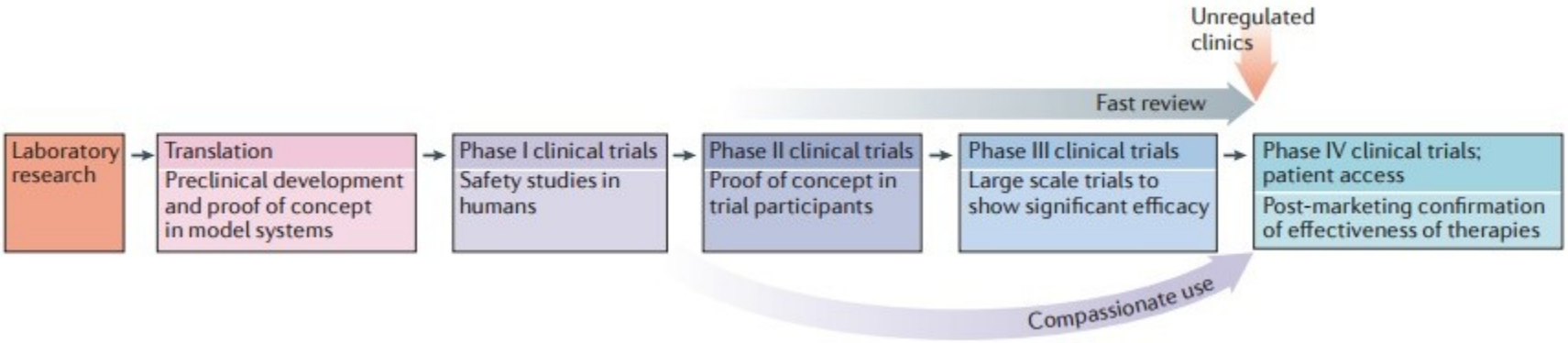
İlacın etkili doz sınırları, klinik etkinliği,biyolojik aktivitesi, yarar ve güvenilirliği az sayıdaki hastada araştırılır. Bu aşamada optimum doz ve doz aralıkları hesaplanılır. Bu fazın ana amacı etkinlik ve güvenirliliktir.

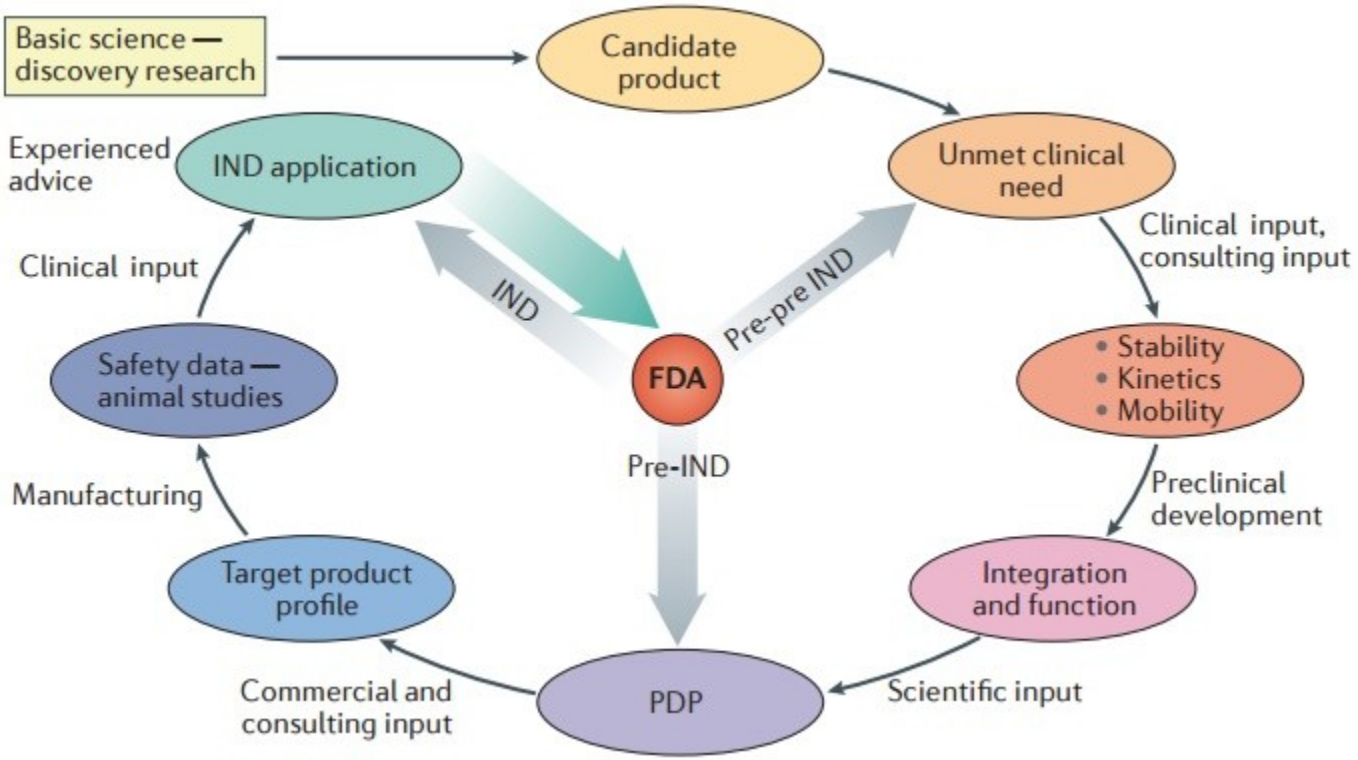
Faz 3 Çalışmalar;

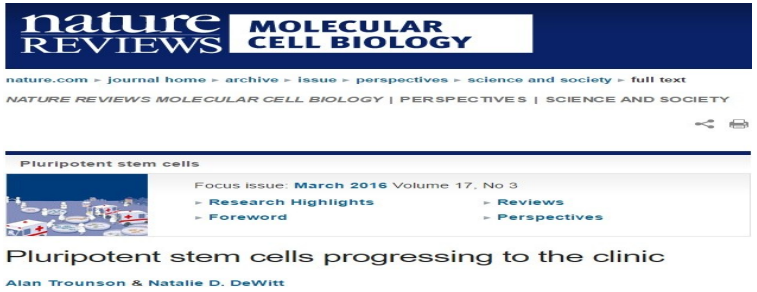
Birinci ve ikinci aşamayı geçen ilaçlar daha geniş bir populasyonda denenir ve plasebo kontrollü çalışmalarla güvenilirliği, karşılaştırmalı çalışmalarla etkinliği araştırılır. Fazın ana amacı etkinliğin kanıtlanması ve yan etkilerin izlenmesidir.

Faz 4 Çalışmalar;

İlk 3 aşamayı geçen ilaçlar ruhsat alır ve pazara verilir. İlaç pazara verildikten sonra yapılan her türlü çalışma 4. faza aittir.


Unregulated clinics
Fast review
Laboratory research
Translation
Preclinical development and proof of concept in model systems
Phase I clinical trials
Safety studies in humans
Phase II clinical trials
Proof of concept in trial participants
Phase III clinical trials
Large scale trials to show significant efficacy
Phase IV clinical trials; patient access
Post-marketing confirmation of effectiveness of therapies
Compassionate use


Basic science — discovery research
Candidate product
Experienced advice
IND application
Unmet clinical need
Clinical input, consulting input
Clinical input
IND
Pre-pre IND
Safety data — animal studies
FDA
• Stability
• Kinetics
• Mobility
Pre-IND
Manufacturing
Preclinical development
Target product profile
Integration and function
Commercial and consulting input
PDP
Scientific input



Kök Hücre Tedavisi - Gelecek

- Gelecekte, hücre tedavisi rejeneratif tıbbın majör gereçlerinden olacaktır.
- Bugüne kadar yapılan majör klinik çalışmalarda teratoma insidansı kontrol altına alınmış görünmektedir.
- Rejeneratif tıpta geniş klinik uygulama beklentileri henüz karşılanmadı.
- Başlangıçtaki bazı abartılı beklentiler hayal kırıklığına dönüştü.
- Ses getiren, iyi tasarlanmış, basit ve translasyonel çalışmalar; bu hücrelerin potansiyel terapötik uygulamalarını keşfetmeye devam etmelidir.
- Parasal sorunlar bu çalışmaların devamının temini için majör sorun olmaya devam edecektir.

Kardiyolojide Güncel Kök Hücre Uygulamaları, 2005

Uz. Dr. Mustafa Yıldız

Prof. Dr. Oktay Seymen

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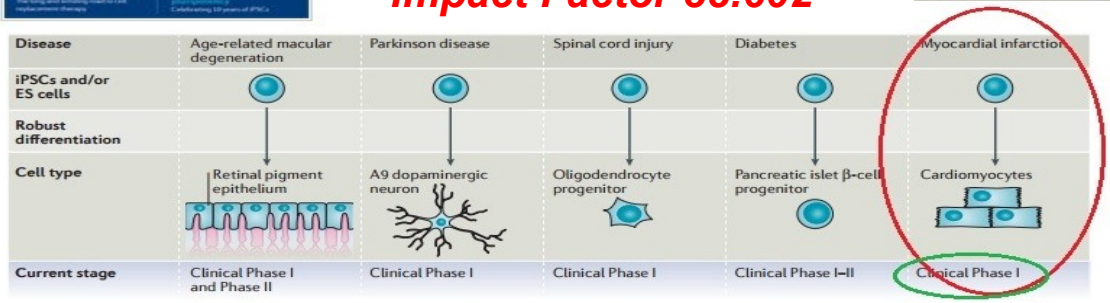

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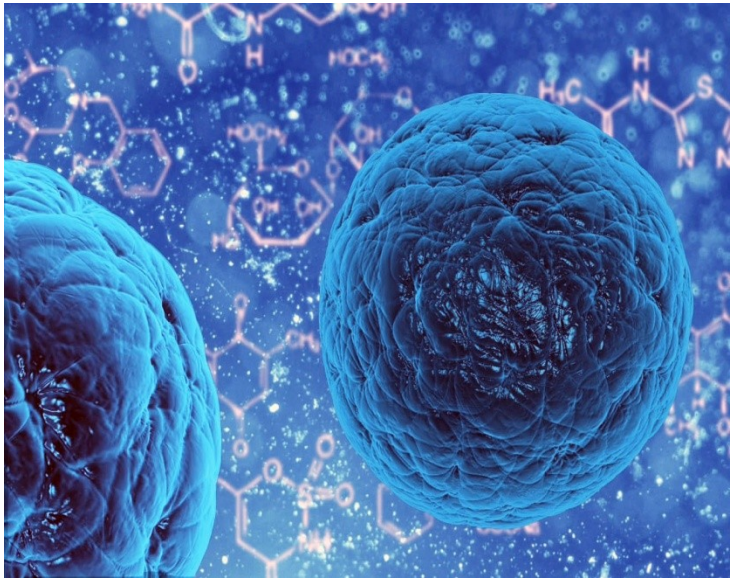

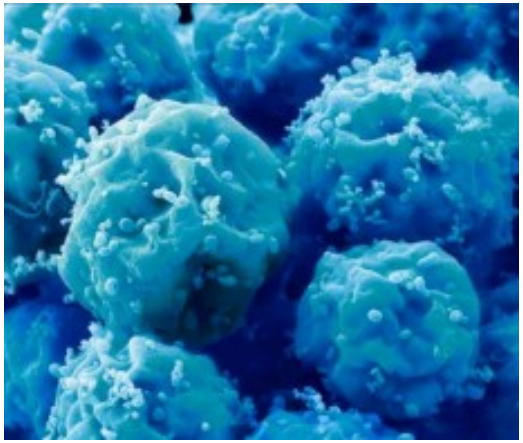

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