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Fate of artificial tissue scaffolds implanted into the rabbit epimyocard

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Fetal and bone marrow stem cells are supposed to be the key players in stem-cell-based therapies. Our aim was to evaluate the potency of two different types of stem cells and their spontaneous behavior *in vivo* after implantation into the epimyocard of healthy rabbit heart ventricles. For this purpose, rabbit allogenic fetal and autologous bone marrow stem cells were seeded on collagen scaffolds and subsequently implanted into the epimyocard of heart ventricles. Two months later these scaffolds were removed and a histological analysis was performed. The results have shown that different types of stem cells have generated two different types of structures in the sites of implantation. Autologous bone marrow cells in the collagen scaffold showed a chondrogenic differentiation pathway. Fetal cells were destroyed by the host immune system, although formation of allogenic structures in the epimyocard was observed, implying that cells of different sources in collagen scaffolds, after implantation *in vivo,* undergo differentiation.

Key words: fetal stem cells, bone marrow stem cells, collagen scaffold, implantation

INTRODUCTION

Mesenchymal stem cells from bone marrow and fetal stem cells from early stage fetuses are adherent stromal cells characterized by the ability to differentiate into mesenchymal tissues such as bone, cartilage, fat and many other types of cells [1, 2]. They have also been shown to suppress immune responses *in vitro* and *in vivo*. Furthermore, results from multiple laboratories suggest that fetal cardiomyocytes can couple functionally with host myocytes, stimulate formation of new blood vessels, and improve myocardial function. The accumulating clinical and experimental evidence indicates that mesenchymal stem cells are promising cell types in the treatment of cardiac dysfunction [1–5]. They may trigger production of reparative growth factors, replace damaged cells and create an environment that favors endogenous car-

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diac repair [6]. Cells alone are hard to apply in certain tissues. Carrier materials – scaffolds – have recently been introduced for tissue regeneration and formation. They mimic cell natural environment and help maintain their phenotype *in vivo*. These extracellular matrix materials have diverse physiological functions by themselves and can also act as reservoirs of cytokines and growth factors, so that they can affect the cell phenotype, attachment, migration and proliferation [7]. The predifferentiated cells receive signals from the environment, thus achieving gradual and complete differentiation. In cell transplantation, survival and integration in the environment of the ischemic myocardium represents a challenge for all types of cells, regardless of their state of differentiation. The strategy is to embed cells in a 3-dimensional structure simulating the extracellular matrix, which is crucial for full tissue restoration and prevention of ventricular remodeling. The clinical translation of cell therapy requires avoidance of potentially harmful drugs and cytokines and a rapid off-the-shelf availability of cells. A combination of predifferentiated cells with a functionalized scaffold, locally releasing molecules aimed to promote the *in-situ* completion of differentiation and improve homing, survival, and function, could be an exciting approach that might circumvent the potentially undesirable effects of growth factor administration and improve tissue restoration [8].

The aim of our experiments was to evaluate the behavior of bone-marrow-derived and fetal stem cells from rabbit, embedded in three-dimensional collagen scaffolds. We implanted these structures into a healthy epimyocard of rabbit heart ventricles and histologically examined them after two months of their persistence *in vivo*.

MATERIALS AND METHODS

Cells

Fetal cells were derived from New Zealand white rabbit embryos at 16–19 days of development. Embryos were removed, minced and treated with collagenase IV (3 mg/ml) in L-15 medium at 37 ºC for 1 h. Later, the cells were centrifuged at 600× g, resuspended in the cultivation medium (DMEM with 20% of FCS and 20 ng of fibroblast growth factor) and cultivated at 37 ºC in 5% CO_2 with antibiotics (penicilline 100 U/ml, streptomycine100 µg/ml). Two days later, the debris were removed and cells spread according to general cell cultivation procedures. Bone marrow stem cells were derived directly by punctation of rabbit bone sacrum using a 18 G needle, a syringe and 0.02 mg/ml heparin solution as an anticoagulant. Bone marrow suspension was centrifuged using a ficoll-paque gradient, and mononuclear cell fractions were placed into T25 tissue flasks in a DMEM medium supplemented with 15% FCS; cells were cultivated at 37 ºC in 5% CO_2 and antibiotics. After 2 weeks of cultivation, only stem cells were left as an adherent culture. These cells were trypsinised (0.02% Trypsin / EDTA), seeded on collagen scaffolds and grown in a bioreactor (Corning spinner flask 250 ml) at 45 rpm.

Scaffolds

Commercially available bovine collagen scaffolds (Southern Lights Biomaterials) were used to seed the cells. Before cell casting, scaffolds had been treated under vacuum conditions to remove all air bubbles. When scaffolds were completely air-bubble-free, they were placed into tissue culture plates (5 cm) covered with 2% low melting agarose and DMEM where niches for implants were formed. Scaffolds were placed in these niches, and cells were gently introduced into them using a syringe and a 21G needle (~$2 \cdot 10^5$ viable cells per scaffold). These cell-scaffold derivatives were grown overnight at 37 ºC in 5% CO_2. Cell persistence in the scaffolds was evaluated the next day, and complete cell–scaffold derivatives were implanted into the epimyocard of heart ventricles.

Animals

A new Zealand white male rabbit was used for implantation experiments. Cell-scaffold derivatives were implanted under general anesthesia into different epimyocard regions of its heart. Fetal cells were implanted into the left ventricle and bone marrow stem cells into the right ventricle. Two months later the heart was removed, and the implantation regions were histologically evaluated.

Histological examination

The results of implantation were analyzed under a microscope. Two months after implantation, slices were stained with hematoxylin / eosin, and their light-microscopic analysis was performed.

RESULTS

Cells and scaffolds

Cell seeding on a collagen scaffold using the organ culture method is fast and simple. Under surgical conditions, collagen scaffolds (Fig. 1) enriched with ~$2 \cdot 10^5$ viable autologous bone marrow cells of an adult rabbit or with rabbit's fetal stem cells were implanted into the epimyocardium of the rabbit's heart ventricle. Cells proliferating in the scaffold cultivated overnight are shown in Fig. 2. The site of the implantation of artificial tissue is shown in Fig. 3. Cells penetrated scaffolds and survived there until implantation.

Cell–scaffold derivatives in the epimyocard of rabbit heart ventricles

Two months after the implantation of artificial tissue, the heart was removed and the implants were evaluated histologically. Our results indicate that bone-marrow-derived stem cells have undergone transformation into chondrocyte-like cells in implantation sites (Fig. 4 A, C). This was confirmed

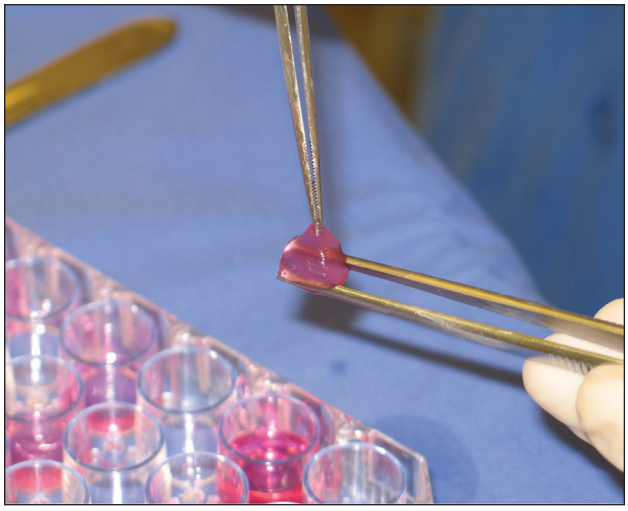

Fig. 1. View of bone marrow embedded cell-scaffold prior to implantation

histologically as the cells shown in Fig. 4 morphologically correspond to mature chondrocytes. The examination of fetal cells containing scaffolds revealed that this type of artificial tissue has evoked the immune reaction towards the implant, but in the period of two months upon implantation, allogenic structures in the pericardium still could be found (Fig. 5 C, D). In histological slices there are no visible signs of viable cells of fetal origin or of any kind of abnormal cells in the analyzed tissue. Although analysis with polarized light showed the presence of allogenic derivatives in the epimyocardial tissue (Fig. 5 D), this could be the result of spontaneous fetal cell differentiation. The calcification found in the site of implantation could be the result of stem cell-scaffold products or of the reaction of the immune system to the implant. Traces of calcification in the histological slices are seen, clearly indicating the rejection of the implant (Fig. 5 A, B).

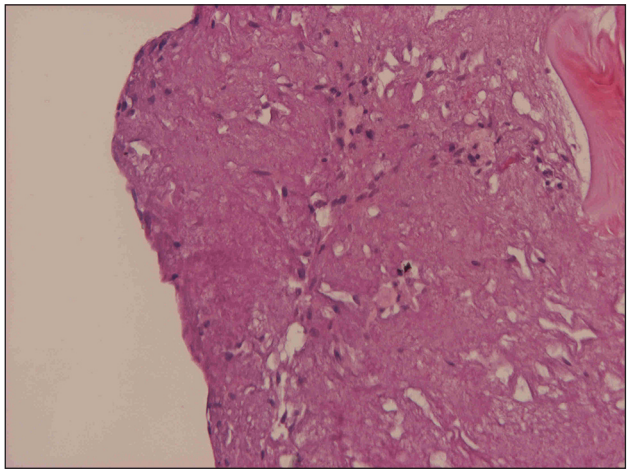

Fig. 2. Stem cells in scaffold. View of histological cuts of collagen scaffold embedded with rabbit bone marrow cells. Hematoxylin / eosin slices were prepared after incubation of cells in organ culture plates overnight. Magnification 100×.

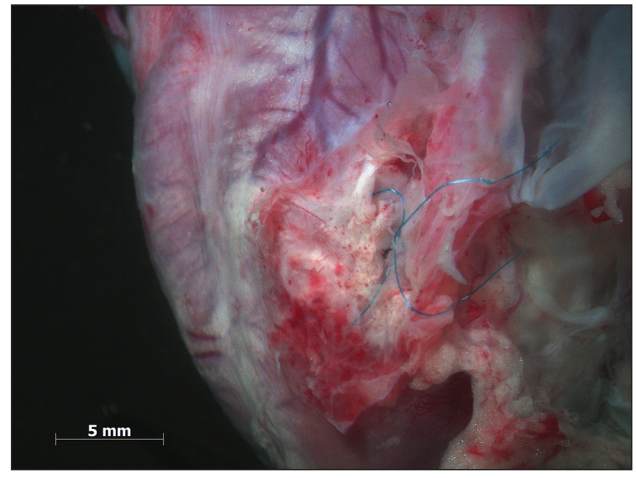

Fig. 3. Fragment of heart with cell-scaffolds implanted into epimyocard of heart ventricles. Two months after implantation. Fetal cell scaffold was implanted into epimyocard using blue surgical suture

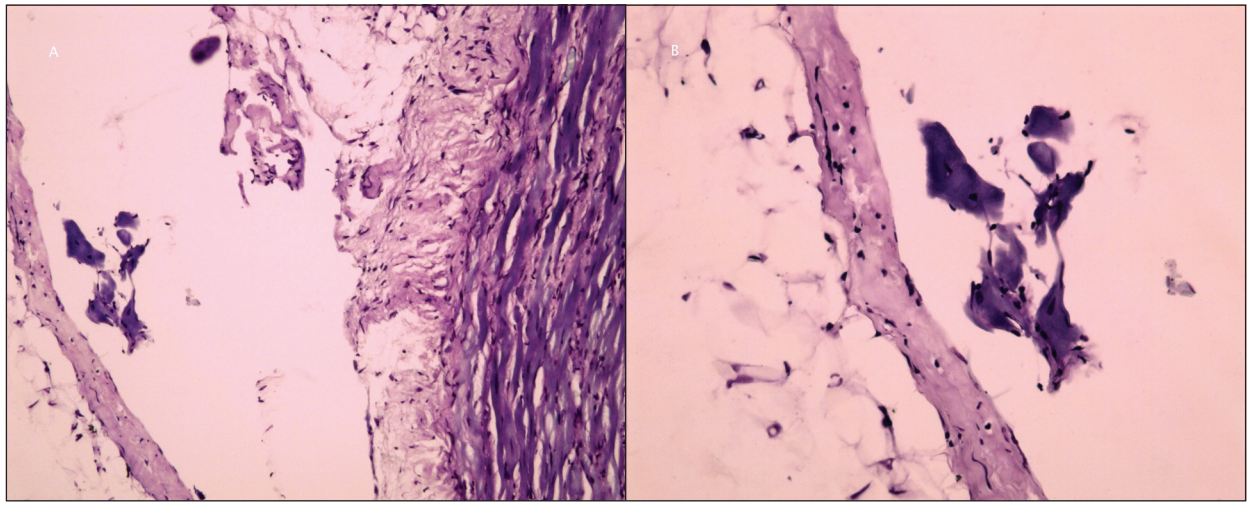

Fig. 4 A, B. Histological view of epimyocard of heart ventricles after bone marrow derived cell-scaffold implantation into rabbit heart.
Cells near pericardiac membrane are described morphologically as chondrocytes. No signs of collagen scaffold are left. Magnification: A – 100×; B – 200×

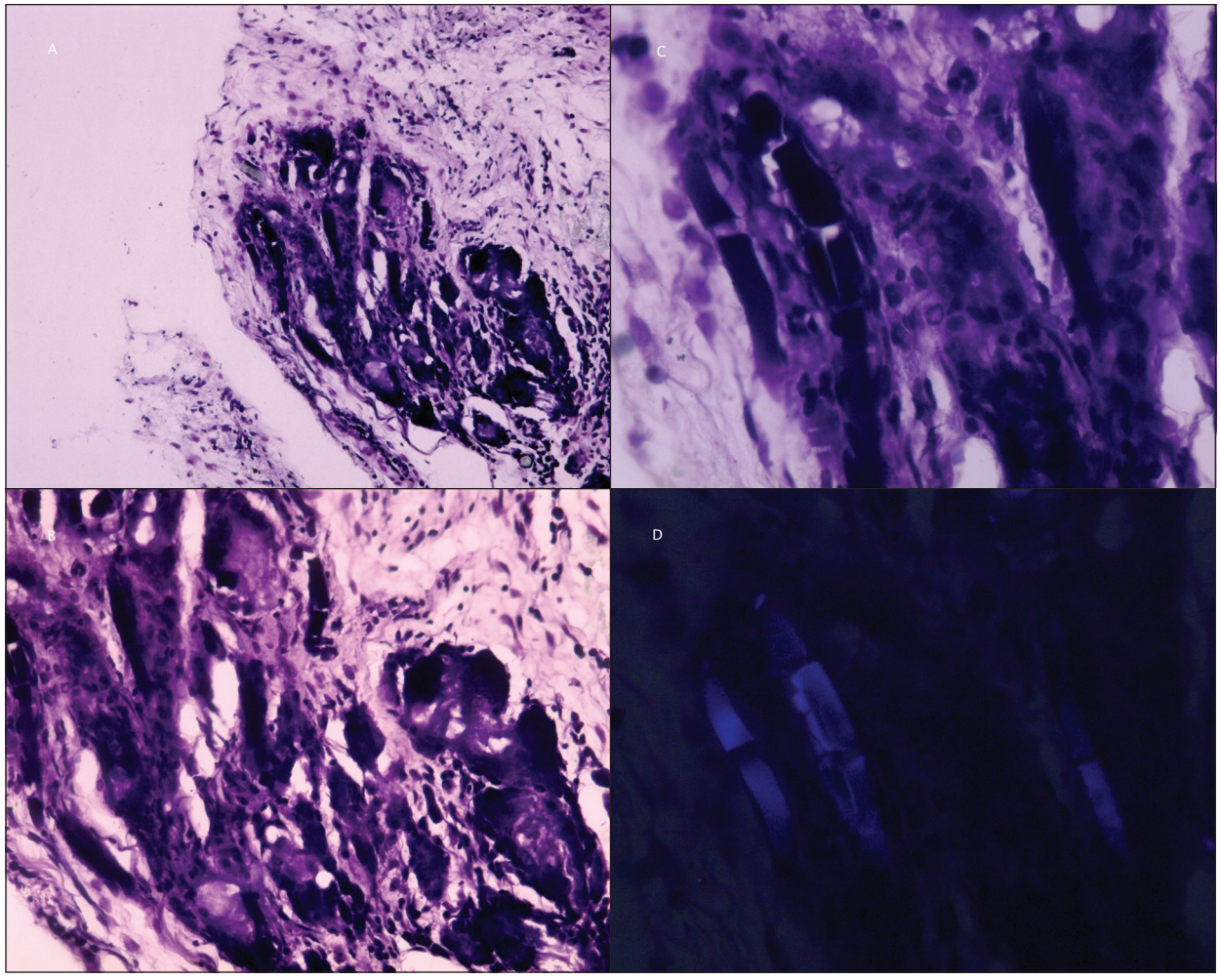

Fig. 5 A, B, C, D. Histological view of epimyocard of heart ventricles after fetal cell-scaffold implantation into rabbit heart. A and B – hematoxylin / eosin cuts of epimyocard with degenerated cell-scaffold; calcification and allogenic structures in the site of implantation are observed. Magnification: A – 100×; B – 200×. C and D – light and polarized light microscopical evaluation of allogenic structures left after fetal cell-scaffold implantation; D – in blue light visible allogenic structures. Magnification 400×

DISCUSSION

The ability of stem cells, in particular from bone marrow, to form bone or cartilage cells in spontaneous differentiation experiments have already been reported [1, 2]. After transplantation of bone marrow cells of adults, it is possible to develop different kinds of tissues [2]. Since the extracellular environment is supposed to determine the fate of stem cells [6–9], we have tested the capability of bone marrow stem cells to generate cardiac tissue by implanting them into epimyocardium of heart ventricles. The histological examination did not reveal any visible rejection of the implanted cells (Fig. 4); it clearly indicated the incidence of spontaneous differentiation and confirmed that bone marrow cells were prone to form bone and cartilage cells [1, 2]. Scaffolds just played the role of a carrier and, as shown in Figs. 4 and 5, were biologically degraded after two months of implantation [10]. It is credible that cardiac tissue could be developed by pre-differentiating these cells into progenitors using the differentiation factors such as VEFG, EGF, IGF [11–13] or enriching the scaffolds with growth factors before seeding the cells [3, 4, 14]. Examination of implanted scaffolds with fetal cells has shown patterns of immune rejection, calcification and formation of allogenic structures. A polarized light image (Fig. 5 D) shows the structures of unidentified allogenic origin, integrated and survived in the site of implantation. According to literature, implantation of allogenic fetal cells is controversial [3, 4, 14]. There are data showing that fetal cells cannot be detected by the host immune system and escape the immune rejection due to their unique immunological properties [4, 15]. In our case, we observed a visible immune reaction towards implanted fetal cells in the scaffold, and two months after implantation no viable cells were detected in the site of implantation. This can be due to the rejection of complete derivative or just an acute reaction of the immune system to fetal cells, because we cannot reject the possibility

of spontaneous differentiation of fetal cells in the scaffolds into allogenic structures visible in Fig. 5. According to published data, fetal cells are able to form spontaneous structures after their implantation [16], and our results confirm that. Our observations suggest that transplantation should be programmed in advance, because the specific extracellular environment around the implant does not always play the expected determinant role.

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References

1. Chanda D, Kumar S, Ponnazhagan S. J Cell Biochem 2010; May 19.
2. Haniffa MA, Collin MP, Buckley CD et al. Haematologica 2009; 94(2): 258–63.
3. Zhang F, Pasumarthi KB. BioDrugs 2008; 22(6): 361–74.
4. Darinskas A, Gasparaviciute R, Malisauskas M et al. Cell Mol Biol Lett 2007; May 10.
5. Biziuleviciene G, Puidokaite G, Siaurys A et al. Int Immunopharmacol 2007; 7(6): 744–9.
6. Kruegel J, Miosge N. Cell Mol Life Sci 2010; Apr 29.
7. Choi KH, Choi BH, Park SR et al. Biomaterials 2010; 31(20): 5355–65.
8. Spadaccio C, Chachques E, Chello M et al. Asian Cardiovasc Thorac Ann 2010; 18(1):79–87.
9. Nesselmann C, Ma N, Bieback K et al. J Cell Mol Med 2008; 12(5B): 1795–810.
10. Ju YM, Choi JS, Atala A et al. Biomaterials 2010; 31(15): 4313–21.
11. Zisa D, Shabbir A, Suzuki G et al. Biochem Biophys Res Commun 2009; 390(3): 834–8.
12. Aghila Rani KG, Kartha CC. Growth Factors 2010; 28(3): 157–65.
13. Khan M, Mohsin S, Khan SN et al. J Cell Mol Med 2009; Dec 11.
14. Roccio M, Goumans MJ, Sluijter JP et al. Panminerva Med 2008; 50(1): 19–30.
15. Moreno R, Martínez-González I, Rosal M et al. Stem Cells Dev 2010; Feb 11.
16. Hentze H, Soong PL, Wang ST. Stem Cell Res 2009; Feb 12.

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DIRBTINIO AUDINIO KARKASŲ, IMPLANTUOTŲ Į TRIUŠIO EPIMIOKARDĄ, IŠLIKIMAS

Santrauka

Darbo tikslas – įvertinti triušio vaisiaus ląstelių ir kaulų čiulpų kamieninių ląstelių, užaugintų ant trijų dimensijų kolageninių karkasų, išgyvenimą ir diferenciacijos kryptį *in vivo* po jų implantacijos ant triušio širdies skilvelių paviršiaus. Vaisiaus ląstelės buvo išskirtos iš 16–19-os dienos Naujosios Zelandijos triušio embrionų ir auginamos DMEM terpėje su 20 % FVS (fetalinis veršelio serumas), 20 ng fibroblastų augimo faktoriumi ir antibiotikais (100 U/ml penicilino, 100 µg/ml streptomicino). Mezenchiminės ląstelės, išskirtos atlikus punkciją į triušio kryžkaulį, auginamos DMEM terpėje su 15 % FVS ir antibiotikais. Eksperimentuose buvo naudojami komerciškai prieinami kolageniniai karkasai (*Southern Lights Biomaterials*). Šie karkasai su autologinėmis kaulų čiulpų ląstelėmis ir embrioninėmis ląstelėmis buvo implantuoti tam pačiam triušiui ant dešinio ir kairiojo širdies skilvelių, dirbtinio audinio fragmentus fiksuojant polipropileno siūlu. Praėjus dviem mėnesiams po implantacijos operacijos triušiui atliekama eutanazija, o išimta širdis laikoma lede. Per tris valandas atliekama histologinė analizė. Mėginiai analizuojami šviesiniu mikroskopu nudažius hematoksilinu / eozinu. Eksperimento rezultatai rodo, kad kaulų čiulpų kamieninės ląstelės širdies audinyje diferencijuojasi į chondrocitus. Vaisiaus ląstelės implantuoto kolageninio karkaso srityje sukelia imuninį atsaką. Analizuojant pavyzdžius poliarizuotoje šviesoje buvo rasta alogeninių struktūrų darinių, ir tai gali būti susiję su spontanine vaisiaus ląstelių diferenciacija. Sukietėjusios struktūros aplink implantacijos zonas (tą patvirtina ir kalcifikacijos pėdsakai histologiniuose preparatuose) galėjo atsirasti dėl kamieninių ląstelių karkasų produktų ar kaip imuninės sistemos atsako rezultatas. Apibendrinant gautus rezultatus, galima daryti išvadas, jog naudoti kolageniniai karkasai atliko tik ląstelių imobilizavimo funkciją ir suiro praėjus dviem mėnesiams po implantacijos. Pritaikant alogenines ir autologines kamienines ląsteles širdies ar kitų audinių regeneracijai, būtina nukreipti ląsteles atitinkama diferenciacijos kryptimi (pvz., paveikiant įvairiais augimo faktoriais), kadangi ekstraląstelinės aplinkos reguliaciniai mechanizmai ne visais atvejais yra pakankami.

Raktažodžiai: fetalinis veršelio serumas, kaulų čiulpų kamieninės ląstelės, kolageniniai karkasai, implantacija