# Kvantifikacija remodelacije miokarda u animalnom modelu infarkta miokarda fazno-kontrastnim oslikavanjem X-zrakama proizvedenim sinkrotronom

# The Quantification of Myocardial remodelling in a Rat Model of Myocardial Infarction by Synchrotron X-ray Phase Contrast Imaging

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**Uvod**: Remodelacija miokarda je skupni naziv za odgovor stanica, tkiva i organa na različite vrste oštećenja, poput infarkta miokarda (IM). Iako se globalna remodelacija lijeve klijetke (LK) može procijeniti ehokardiografijom, magnetskom rezonancijom ili kompjuteriziranom tomografijom srca, niti jedna metoda ne može istovremeno kvantificirati promjene na mikroskopskoj i makroskopskoj razini. Istraživačka tehnika fazno-kontrastnog oslikavanja X-zrakama proizvedenim sinkrotronom (X-PCI) se može koristiti za 3D analizu cijelog organa, ali i kardiomiocita.[1,2] Životinjski modeli glodavaca se često koriste u bazičnim i translacijskim istraživanjima ishemije miokarda.

**Metode**: IM je izazvan podvezivanjem lijeve koronarne arterije lijevostranom torakotomijom u mladih odraslih Wister štakora (8-11 tjedana starosti). Štakori su žrtvovani 2 tjedna nakon zahvata, a srca su oslikana X-PCI tehnikom (TOMCAT zraka, Paul Scherrer Insitut, Švicarska koristeći energiju od 20keV s dvije veličine voksela u određenim regijama srca. Snimke dobivene ovom tehnikom korištene su za izračun dimenzija LK, mase miokarda, analizu orijentacije miokardnih vlakana, te analizu pojedinačnih kardiomiocita (izračun površine poprečnog presjeka (PPP) kardiomiocita). Analizirana su 4 srca nakon izazvanoga IM, te jedno kontrolno srce zdravog štakora. U 2 srca nakon izazvanoga IM analizirali smo kardiomiocite u području samog IM (kardiomiociti neposredno uz fibrotično tkivo na mjestu infarkta), te u kontralateralnom području. Odgovarajuća područja su analizirana i u kontrolnom štakorskom srcu. PPP kardiomiocita prikazana je kao prosječna vrijednost sa standardnom devijacijom 10 kardiomiocita u odabranom području miokarda.

**Rezultati**: Dimenzije LK i masa miokarda su prikazani u **tablici 1**. Rezultati izračuna PPP kardiomiocita (**tablica 1**) upućuju na značajnu razliku ($p<0.001$) između kardiomiocita u neposrednoj blizini infarktom zahvaćenog miokarda i kontralateralnih područja LK, kao i u usporedbi s istim područjima kontrolnog srca. Navedeno upućuje na izraženu kompenzatornu hipertrofiju kardiomiocita u neposrednoj blizini IM u usporedbi s kontralateralnim područjem.

**Zaključak**: Rezultati dobiveni pomoću X-PCI su u skladu s dosadašnjim istraživanjima, no predstavljaju X-PCI kao

**Background**: Cardiac remodelling is a set of cellular, tissue and organ changes that develop as a consequence of various injuries to the heart, such as myocardial infarction (MI). Global remodelling can be assessed by echocardiography, magnetic resonance or computed tomography imaging, however combining information on both cellular and entire organ level is still not possible by currently available imaging techniques.[1,2] A prominent technique under research is Synchrotron X-ray Phase Contrast Imaging (X-PCI) that can be used for both 3D analysis of whole hearts, as well as cardiomyocytes (CMCs) without tissue processing or destruction. In basic and translational science rodent animal models are frequently used for myocardial ischemia research.

**Methods**: MI was induced by LAD ligation via a left thoracotomy in an established model of adult (8-11 week-old) Wister rats. The animals were sacrificed after 2 weeks when the hearts were extracted and imaged by X-PCI at TOMCAT beamline (Swiss Light Source, Paul Scherer Institute, Switzerland) using an energy of 20 keV with two different voxel sizes in selected regions of interest. 3D datasets obtained by this technique allowed calculation of ventricular volumes, mass and cavity dimensions, fibre orientation analysis, as well as analysis of individual cardiomyocytes (cross sectional area (CSA) calculation). We quantified global left ventricular (LV) remodelling in 4 post-MI rat hearts, and in a control healthy rat heart. In 2 post-MI hearts, the cardiomyocytes were analysed in the area of the MI (preserved cells adjacent to the fibrotic post-MI myocardium - peri-MI zone), and in the contralateral region (the non-affected myocardium). Cardiomyocytes of corresponding areas were analysed in the healthy heart alike. CSA was expressed as the mean value with standard deviation of measurements of 10 CMCs per area.

**Results**: **Table 1** shows indices of global myocardial remodelling confirming wall thinning and increase in size and mass of the LV in post-MI rat hearts. The results of CSA calculations (**Table 1**) indicate significant ($p<0.001$) differences in CSA between peri-MI and non-MI areas of ischemic hearts, as well as compared to the healthy rat, indicating compensatory hypertrophy pronounced in the peri-MI area as opposed to contralateral region.

**Conclusion**: X-PCI provides results consistent with previous research in the field but obtained by one single tech-

**TABLE 1. Myocardial (global) and cellular remodelling.**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Myocardial (global) remodelling** | | | | | | |
| | **Post MI Hearts Average** | MI Heart 1 | MI Heart 2 | MI Heart 3 | MI Heart 4 | **Healthy Heart** |
| **Wall thickness (mm)** | **1.8±0.8** | 1.9±0.9 | 2.1±0.9 | 1.8±0.7 | 1.5±0.7 | **2.2±0.4** |
| **LV cavity volume (ml)** | **0.26±0.090** | 0.20 | 0.20 | 0.23 | 0.39 | **0.11** |
| **LV volume (ml)** | **0.62±0.02** | 0.62 | 0.63 | 0.60 | 0.63 | **0.58** |
| **LV mass (g)** | **0.67±0.03** | 0.71 | 0.67 | 0.63 | 0.66 | **0.61** |
| **Cellular remodelling** | | | | | | |
| | **Post-MI rat Average** | MI Heart 1 | MI Heart 2 | - | - | **Healthy rat** |
| **Cardiomyocyte cross sectional area/ μm²** | | | | | | |
| **Contralateral region** | **499±141** | **477±116*** | **521±160*** | - | - | **312±62** |
| **Peri-MI zone** | **737±127** | **773±118** | **701±126** | - | - | **330±92** |
| **Overall** | **321±75** | **625±194ϕ** | **611±168ϕ** | - | - | **-** |

*marks significant difference in cardiomyocyte cross sectional areas between different myocardial areas in the same rat heart, while ϕ marks significant difference in cardiomyocyte cross sectional area between same myocardial areas of affected and non-affected hearts (p≤0.01).

MI - myocardial infarction, LV - left ventricle

jedinstvenu tehniku istovremene procjene kako globalne remodelacije LK, tako i hipertrofije na staničnoj razini.

nique that proves it valuable for quantifying both global and cellular myocardial remodelling.

## LITERATURE

1. Dejea H, Garcia-Canadilla P, Stampanoni M, Zamora M, Crispi M, Bijnens B, et al. Microstructural Analysis of Cardiac Endomyocardial Biopsies with Synchrotron Radiation-Based X-Ray Phase Contrast Imaging. In: Pop M, Wright GA. Functional Imaging and Modelling of the Heart. Cham: Springer; 2017. 23-31.
2. Gonzalez-Tendero A, Zhang C, Balicevic V, Cárdenes R, Loncaric S, Butakoff C, et al. Whole heart detailed and quantitative anatomy, myofibre structure and vasculature from X-ray phase-contrast synchrotron radiation-based micro computed tomography. Eur Heart J Cardiovasc Imaging. 2017 Jul 1;18(7):732-741. https://doi.org/10.1093/ehjci/jew314