Promotion of Myocardial Repair by Dual Delivery of IGF-1 and HGF from Injectable Alginate Biomaterial

Emil Ruvinov[1], Jonathan Leor[2], Smadar Cohen[1]

[1]Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel, [2]Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer, Israel.

Introduction: Various factors are known to improve tissue salvage and elicit a favorable course of tissue repair after myocardial infarction (MI)**.** However**,** the proper spatio-temporal delivery of these factors to infarcted hearts represents a challenge**.** We hypothesized that dual delivery of insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) by injectable affinity-binding alginate biomaterial would maximize their therapeutic effects, leading to a more favorable course of tissue restoration after acute MI.

Methods: Bioconjugation of alginate-sulfate with growth factors were achieved by incubation of the components at 37ºC for 1 h. For release studies, the affinity-binding (alginate-sulfate-growth factor bioconjugates containing) system was produced as hydrogel microbeads. To confirm the bioactivity of the released proteins, the release medium was added to isolated rat neonatal cardiac cultures. *In vivo*, short (1 week) and long-(4 and 8 weeks) term effects of dual (IGF-1/HGF) delivery by injectable affinity-binding alginate were tested in a rat model of acute MI. Morphometric parameters and fibrosis of the left ventricle (LV) were evaluated using Masson's Trichrome staining. Angiogenesis, cell apoptosis, cell proliferation and the expression of cardiomyogenic markers was detected by immunohistochemistry. Cardiac function and remodeling were evaluated by 2D echocardiography.

Results: MALDI-TOF mass spectrometry analysis showed that bioconjugation of IGF-1 or HGF with alginate-sulfate protects the proteins from trypsin proteolysis. The release profile from affinity-binding alginate hydrogel revealed a sequential release pattern (IGF-1 followed by HGF)**,** for 1 week. Factor bioactivity was confirmed by Western blot analysis for induced phosphorylation of AKT and ERK1/2 for IGF-1 and HGF, respectively, and by prevention of cell death in H_2O_2–induced oxidative stress model in cardiac cell cultures. In acute MI model, 4 weeks after biomaterial injection, the treatment with sequentially-delivered proteins increased scar thickness, prevented infarct expansion and reduced fibrosis. This treatment also increased mature (α-SMA-positive) blood vessel density and area. Active caspase-3 staining showed that dual delivery of IGF-1/HGF also reduced apoptosis at the infarct. These therapeutic effects were preserved for a period of at least 8 weeks after treatment, when compared to soluble proteins-treated animals (Fig. 1). The sequential delivery of IGF-1/HGF induced the greatest number of Ki-67-positive myocytes, 1 week after treatment, and also resulted in higher incidence of GATA-4 positive cell clusters at 4 weeks.

2D echocardiography showed that IGF-1/HGF in affinity-binding alginate prevented LV dilatation, as shown by attenuated increase in LV end systolic and diastolic diameters, when compared to the treatment with soluble factors, 4 weeks after MI/injection. Preservation of LV size in IGF-1/HGF-affinity-bound alginate-treated animals was also evident 8 weeks after injection (Fig. 2).

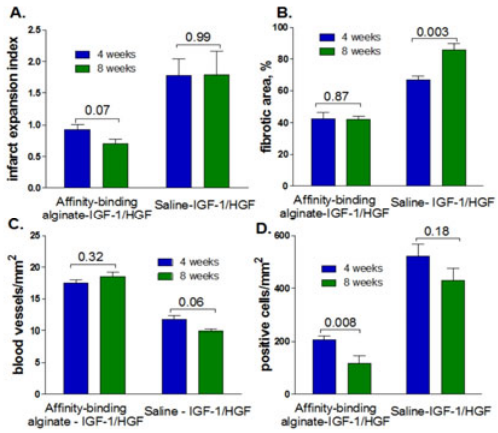

Figure 1. Therapeutic effects of the sequential IGF-1/HGF delivery are preserved for 8 weeks after MI. A. Reduction in infarct expansion; B. Preserved fibrotic area; C. Preserved blood vessel density; D. Reduction in apoptotic cell number.

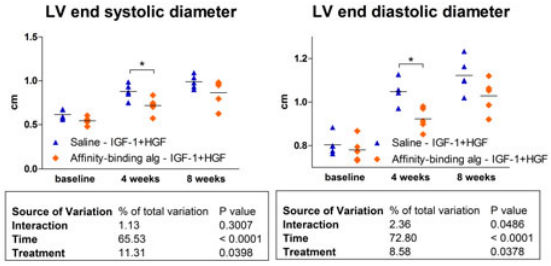

Figure 2. The effect of sequential IGF-1/HGF delivery on LV remodeling, 4 and 8 weeks after MI.

Conclusions: The dual delivery of IGF-1/HGF from alginate biomaterial represents an applicable strategy to treat MI. The affinity-binding mechanism preserved factor activity and enabled its protection in the harsh MI environment. This treatment also showed a marked long-term therapeutic efficacy at various levels, including prevention of adverse LV remodeling, induced angiogenesis and improved cell survival. Finally, the developed approach showed a potential to induce endogenous regeneration of cardiac muscle.