Integration of Multiple Cell-Matrix Interactions into Alginate Scaffolds for Cardiac Tissue Regeneration

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Introduction

Engineering a functional cardiac tissue *in vitro* is one of the most challenging tasks for tissue engineers. In this research, we aimed to reconstruct the microenvironment promoting cardiac tissue regeneration by presenting multiple cell-matrix interactions, in a similar manner to their presentation by the extra-cellular matrix *in vivo*. Thus, two fibronectin-derived peptides (RGD and heparin binding peptide (HBP) were bound into alginate scaffold, mimicking the specific interactions of ECM with integrin and syndecan on cell membrane, respectively

Materials and Methods

The peptides (G_4RGDY, G_4SPPRRARVTY or their combination) were covalently-attached to alginate via the carbodiimide chemistry[1], creating an amide bond between the terminal amine of peptide and the alginate carboxylic group. The influence of peptide binding on scaffold internal morphology (e.g., porosity) and stiffness were evaluated by Scanning Electron Microcopy and mechanical testing. Neonatal rat cardiac cells were isolated and cultured as described[2]. Cardiac cell-scaffold interactions and tissue organization and maturation were evaluated by confocal microscopy and immunohistochemistry of resulting constructs. AKT activation and expression patterns of cardiac proteins (such as alpha-actinin, Cx-43, N-Cad) in the regenerated cardiac tissue were evaluated by Western Blotting.

Results

Our results show that alginate modification with either one of the peptides or their combination had no measurable effect on the porosity and stiffness of the resulting scaffolds. Cardiac cell cultivation within the HBP/RGD-modified scaffold was favorable compared to that in single peptide- or unmodified alginate scaffolds. The increased AKT phosphorylation in the cell constructs in HBP/RGD-modified scaffolds (data not shown) suggests adhesion-dependant pathway activation and pro-survival signaling provided by the adhesion peptides. Already by 7, well-developed myofibers with distinguished striation were observed in HBP/RGD scaffold (Fig. 1). In the RGD-attached scaffold, sporadic islands of striation were seen, but no developed myofibers. In contrast, the HBP-modified and unmodified scaffolds had no such as effect on cardiac reorganization. Finally, alpha-actinin, Connexin-43 and N-Cadherin expression profiles presented better tissue maturation and regeneration of a functional cardiac muscle tissue within the scaffolds with the multiple functional cues (Fig. 2).

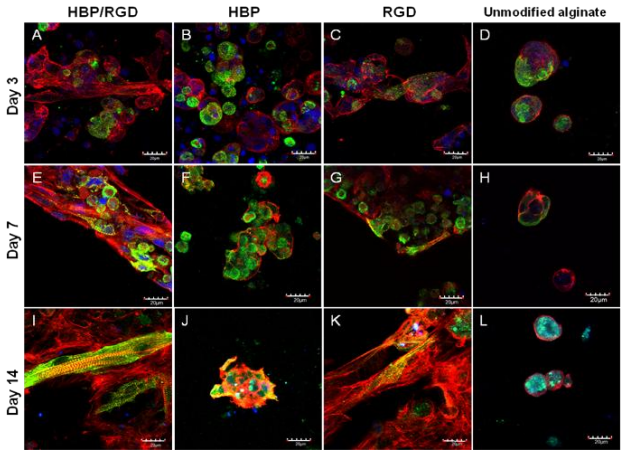

Fig 1. Confocal images of cardiac constructs in HBP/RGD, HBP-, RGD-modified and unmodified scaffolds at different times during cultivation. Cardiac cells are stained for F-actin, sarcomeric alpha-actinin and nuclei

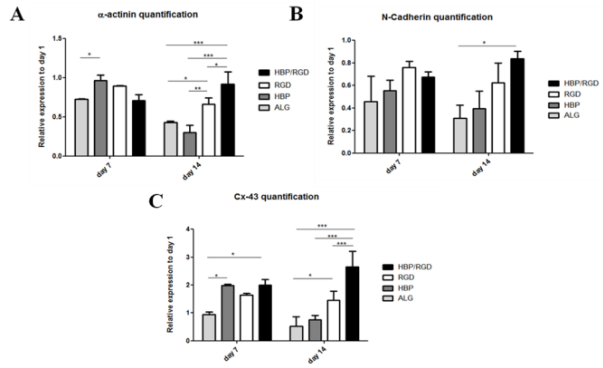

Fig 2. Expression of representative cardiomyocyte proteins by Western Blot**.** The relative folds of increase in the contractile protein a-sarcomeric actinin (A), the cell-cell adhesion molecule N-Cadherin of the intercalated disc (B) and the gap junction protein Connexin-43 (C).Asterisks denote significant difference (2-way ANOVA), when *p<0.05, **p<0.01 and ***p<0.005.

Discussion and Conclusions

Our data establish the potential use of HBP/RGD alginate scaffolds as a better ECM-mimicking microenvironment for inducing regeneration of functional cardiac tissue, in vitro.

References

1. Tsur-Gang O, Ruvinov E, Landa N, Holbova R, Feinberg M, Leor J, Cohen S (2009) **Biomaterials**, 30(2):189-195
2. Dvir T, Kedem A, Ruvinov E, Levy O, Freeman I, Landa N, Holbova R, Feinberg MS, Dror S, Etzion Y, Leor J, Cohen S. (2009) **Proc. Natl. Acad. Sci. (PNAS, USA)**, 106 (35): 14990-14995.