Hyaluronan based scaffold for cardiomyocyte adhesion

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Introduction

Hyaluronic acid (HA) is known as a linear polysaccharide consisting of β -(1-4)-d-sodiumglucuronate- β -(1-3)-N-acetyl-d-glucosamine units. Hyaluronan derivatives are often used in biomedical applications as substrates for tissue engineering [1]. Peptide with active sequence YIGSR enabling adhesion of cardiomyocytes was chosen for this study.

Methods and Results

Non-woven textile fabrication

The HA non-woven fabrics were formed using wet spinning technology that had been applied for hyaluronic acid (Mw 1 MDa, Contipro a.s., Czech Republic) [2]. Non-woven fabrics with basis weight in the range of 15 and 60 g.m⁻² were prepared from staple fibers with typical diameter of 1 μ m.

Figure 1: Non-woven textile (1×1 cm) – SEM image showing inner structure of non-woven textile (left) and macroscopic view of the same scaffold (right).

Solid Phase Peptide Synthesis using HA carrier

Couplings were performed in DMF where 3 molar excess of Fmoc amino acid and DIC/OxymaPure as coupling agents were employed for 2 h. N-terminal Fmoc group was removed with 20% piperidine in DMF. HA carrier

bearing Fmoc protected peptide was used for the determination of fabric substitution with the aid of common Fmoc release test. Substitution of non-woven fabrics varied in the range $0.19 - 0.21 \text{ mmol.g}^{-1}$.

Approaches for synthesis or attachment of peptides to hyaluronan-based materials (fibers and textile scaffold) have been developed [3,4]. Fully protected peptides were assembled on Wang resin and after deprotection and splitting they were coupled to HA fibers. The structure of peptides was checked by amino acid analysis and HPLC.

Structure of applied peptides:

A H-Gly-Gly-Glu-Gly-Tyr-Gly-Glu-Gly-Tyr-Ile-Ser-Arg-Ahx-Ahx-Nle-OH

B H-Ile-Lys-Val-Ala-Val-Ahx-Ahx-Nle-OH

C H-Gly-Gly-Glu-Gly-Tyr-Gly-Glu-Gly-Ile-Lys-Val-Ala-Val-Ahx-Ahx-Nle-OH

D H-Ahx-Ahx-Nle-OH

Cardiomyocyte adhesion

Stem cells pre-differentiated to cardiomyocytes were stained by DiI (15 min, $E_{x_{\max}}/E_{m_{\max}}$ 514/570 nm) prior to seeding to UV-sterilized non-woven textile previously soaked in cultivation medium (10^5 cells/scaffold ca. 1.5 cm^2). The adhesion of cells was monitored during the 7-day long cultivation.

Figure 2: Stem cells pre-differentiated to cardiomyocyte and cultivated on non-woven textile with different conjugated peptides. Time points 1 day and 7 days are displayed. The scale bar unites are μm . Cells are stained by DiI (red).

Discussion

Scaffolds were stable under the cultivation conditions. Stem cells adhered uniformly on the non-woven HA biofunctionalized by YIGSR peptide, which should support the adhesion of cardiac myocytes (Fig. 2-A). Contrary, cardiomyocytes attached poorly and unequally, forming cell clumps on the textile modified by IKVAV peptide (Fig 2-B), which is not primarily used to enhance cardiomyocytes adhesion. Controls confirmed that the adhesion was not supported by the peptide used ahead of the sequence YIGSR (Fig. 2-C) as well as cells did

not adhere on the textile with Ahx linkers used to anchor the adhesive motives to HA. These data indicate that the activity of YIGSR peptide was maintained, and its functional group was accessible for the cellular integrins enabling the focal adhesion. The negative results obtained with IKVAV peptide supported the selectivity and specificity for this process.

Conclusion

Preparation method employing the non-woven fabrics of HA as solid support has been studied with different short peptides specifically enabling adhesion of cardiomyocytes. Biocompatibility and biodegradability of the HA carrier in human body was considered as the main advantage. Such a kind of HA modification may improve the properties of wound healing materials because it enables to eliminate the need of its removal after surgery. Preliminary results and biological effects of the prepared peptide models confirmed the rightness of our approach.

References

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