Diferensiasi Osteogenik dari Sel Punca Mesenkim yang Dikultur pada Perancah Hibrida Polivinil Alkohol (PVA) dan Fibroblast-Derived Matrix (HFDM) Asal Manusia = Osteogenic Differentiation of Mesenchymal Stem Cells Cultured on Polyvinyl Alcohol and Human Fibroblast-Derived Matrix Hybrid Scaffold

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Abstrak

Diferensiasi osteogenik dari Sel punca mesenkim (MSC) menjadi osteoblas memiliki signifikansi klinis yang sangat penting untuk mengobati cedera tulang. Berbagai penelitian telah dilakukan untuk meneliti faktor-faktor yang dapat meningkatkan diferensiasi osteogenik, termasuk pengembangan perancah untuk kultur MSC. Perancah Polivinil Alkohol (PVA)/ Fibroblast-derived Matrix (hFDM) asal manusia menjadi salah satu kandidat perancah yang diduga dapat mendukung diferensiasi osteogenik MSC. Keberadaan matriks ekstraseluler (ECM) pada perancah dapat meregulasi berbagai aktivitas seluler melalui komponen protein matriks yang terdapat pada ECM. Protein matriks berperan sebagai sekuesterasi berbagai faktor pertumbuhan. Faktor pertumbuhan seperti BMP2 dan chordin diketahui dapat meregulasi diferensiasi osteogenik. Proses terjadinya diferensiasi osteogenik dapat diamati melalui akumulasi mineral kalsium yang terdeposit pada matriks ekstraseluler. Tujuan dari penelitian ini adalah untuk mengetahui metode optimum dalam pembuatan perancah PVA/hFDM, danmengetahui peran perancah PVA/hFDM dalam mempengaruhi diferensiasi osteogenik MSC dengan mengukur ekspresi gen BMP2, dan chordin, serta ekspresi kadar kalsium relatif pada matriks ekstraseluler. Optimasi pembuatan perancah PVA hFDM dimulai dengan optimasi medium kultur, waktu kultur, preparasi, dan teknik deselulerisasi. hFDM dikarakterisasi menggunakan pewarnaan Hematoxylin, Masson Trichrome, dan Imunohistokimia untuk mengetahui keberadaan protein matriks. MSC dikultur pada perancah PVA/hFDM untuk uji diferensiasi osteogenik selama 21 hari. Sampel RNA diisolasi pada hari ke-7,14, dan 21. Ekspresi gen BMP2 dan chordin dianalisis menggunakan metode qRT-PCR. Adapun ekspresi kadar kalsium relatif dianalisis dengan uji kualitatif dan kuantitatif pewarnaan Alizarin Red. Hasil penelitian ini menunjukkan protokol pembuatan perancah PVA/hFDM telah dioptimasi, dan karakterisasi hFDM memperlihatkan keberadaan protein matriks berupa kolagen dan biglycan. Ekspresi gen BMP2 menurun pada kelompok MSC yang dikultur pada perancah PVA/hFDM baik di hari ke-7, 14, dan 21. Sedangkan ekspresi gen chordin meningkat pada kelompok MSC yang dikultur pada perancah PVA/hFDM di hari ke 7, dan 14, kembali menurun di hari ke-21. Ekspresi kadar kalsium relatif cenderung meningkat pada kelompok MSC yang dikultur pada perancah PVA/hFDM dengan gambaran mikroskopis berupa bercak merah pada permukaan perancah. Kesimpulan dari penelitian ini adalah perancah PVA/hFDM cenderung dapat mendukung diferensiasi osteogenik MSC. Hasil penelitian menunjukkan bahwa penggunaan perancah PVA/hFDM dapat menurunkan ekspresi gen BMP2, dan meningkatkan ekspresi gen chordin, serta cenderung meningkatkan ekspresi kadar kalsium relatif yang terdeposit pada matriks ekstraseluler.

.....Osteogenic differentiation from Mesenchymal Stem Cell (MSC) to osteoblasts has great clinical significance for treating bone injury. Various studies have been conducted to investigate factors that can enhance osteogenic differentiation, including scaffold development for MSC culture. Scaffold Polyvinyl

Alcohol (PVA) / human Fibroblast-derived Matrix (hFDM) is a scaffold candidate assumed to support osteogenic differentiation of MSCs. The extracellular matrix (ECM) presence on the scaffold can regulate various cellular activities through the matrix protein components contained in the ECM. Matrix protein plays a role in sequestering multiple growth factors. Growth factors such as BMP-2 and chordin are to regulate osteogenic differentiation. The process of osteogenic differentiation can be observed by accumulating calcium minerals in the extracellular matrix. The purpose of this study was to determine the optimal method for making PVA / hFDM scaffold and to determine the role of the PVA / hFDM scaffold in affecting MSC osteogenic differentiation by measuring the expression of BMP2 and chordin genes, as well as the expression of relative calcium levels in the extracellular matrix. Optimization of making hFDM PVA scaffold begins with the optimization of culture medium, culture time, preparation, and decellularization techniques. hFDM was characterized using Hematoxylin, Masson Trichrome, and Immunohistochemical staining to determine matrix proteins' presence. MSCs were cultured on the PVA / hFDM scaffold for osteogenic differentiation assay for 21 days. RNA samples were isolated on day 7,14 and 21. Expression of BMP2 and chordin genes were analyzed using the qRT-PCR method. The expression of relative calcium levels was analyzed by qualitative and quantitative tests of Alizarin Red staining. The results of this study indicate that the PVA / hFDM scaffold preparation protocol has been optimized, and the hFDM characterization shows the presence of matrix proteins in the form of collagen and biglycan. BMP-2 gene expression decreased in the MSC group cultured on the PVA / hFDM scaffold on days 7, 14, and 21. In contrast, the chordin gene expression increased in the MSC group cultured on the PVA / hFDM scaffold on days 7, and 14, back down on day 21. The expression of relative calcium levels tended to increase in the MSC group cultured on the PVA / hFDM scaffold with a microscopic appearance of red spots on the scaffold surface. This study concludes that Scaffold PVA / hFDM tends to support osteogenic differentiation of MSCs. The results showed that the use of the PVA / hFDM scaffold could decrease the expression of the BMP2 gene, and increase the expression of the chordin gene, and tended to increase the expression of the relative calcium levels deposited in the extracellular matrix.