594d Biomimetic Interfaces Using Cell-Specific, Phage-Display-Selected Peptides

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Despite the recent advances towards the development of biomimetic materials for tissue engineering applications, the design of bioactive constructs for specific cell types still remains a challenge. To address this problem we performed phage display screening to identify new ligands eliciting specific cellular responses and directing new tissue formation mediated by specific interactions. We report the selection of ligand based peptide sequences that are highly specific for cultured endothelial progenitor cells (EPC). It has been shown recently that EPC isolated from peripheral blood and expanded in-vitro provide a suitable source for constructing functional tissue engineered scaffolds. Although the number of EPC in adult peripheral blood is low, it has been shown that when isolated and expanded in culture, EPC can undergo more than 1000 population doublings. This doubling ability is in contrast to that of mature endothelial cells that can be grown in culture but senescence after about 30 population doublings. The discovery of new peptide ligands for cell specific types promises to be valuable tool both in research and clinical applications. Our biopanning strategy for isolation of ligand peptides for cell surface receptors is designed to allow using whole cells as an affinity matrix. This approach has the great advantage that the receptors are more likely to be in their native conformation with all their natural post translational modifications and that neither purification nor prior knowledge of a particular receptor is required. An additional strength of this approach is that it is highly inductive in that it does not rely on knowledge of which surface molecules are present, in what concentration and with what specificity. To avoid non specific binding, a negative-positive approach has been included in our experimental protocol. PhD-12 peptide phage display system (New England BioLabs, Beverly, MA) was used for the biopanning experiments. The complexity of the library is in excess of two billion binding clones. To eliminate phage with binding capacity to common receptors the library was pre-adsorbed on human umbilical vein endothelial cells (HUVEC) prior to biopanning with EPC-derived endothelial cells. EPC were isolated from peripheral blood (anticoagulated with 3.8% sodium citrate) using a density gradient centrifugation protocol and subsequently cultured in differentiating medium (EGM-2 and Single Quotes, Clonetics, Inc. San Diego, CA). In the first set of biopanning, the pre-adsorbed phage library was incubated with EPC outgrowth cell suspension in EGM-2 for 1hour. The unbound phage clones were separated from the cell bound phage complexes by centrifugation. After extensive washing, the unbound phage was eluted and amplified in E-Coli culture. An aliquot of the amplified phage was subsequently re-applied to newly trypsinized cells for a total of three biopanning rounds and two amplification steps. After the third round the phage DNA was sequenced and the ligand peptides were deduced after translation. To demonstrate the utility of the phage display selected, EPC-specific peptide sequences, free peptides have been synthesized and covalently immobilized to synthetic surfaces to design biomimetic materials for tissue engineering applications.