Collagen-Mimetic Peptides (CMPs) for Integrin-specific Cellular Recognition and Tissue Engineering

Shih Tak Khew* and Yen Wah Tong*+
**Department of Chemical & Biomolecular Engineering, National University of Singapore*
+Division of Bioengineering, National University of Singapore
10 Kent Ridge Crescent, Singapore 119260

Abstract

This study demonstrated the significant progress in the use of a collagen-mimetic peptide (CMP) for its ability to elicit specific cellular responses, particularly integrin-mediated cell adhesion and signaling pathways. In this study, CMPs containing residues 502-507 (Glycine-Phenylalanine-Hydroxyproline-Glycine-Glutamate-Arginine) (GFOGER) of collagen α_1 (I) were tested for interactions with Hep3B liver cells. Recognition of these biomolecules by the cells appeared conformation-dependent, with the CMP1 of higher triple helix stability being preferred. Absence of the GFOGER hexapeptide in the CMP1' and CMP2' caused an adverse effect on the level of cell adhesion (< 10%). The GFOGER-containing triple helical CMPs effectively inhibited adhesion of Hep3B cells to collagen in a competition assay, suggesting the cell binding to these CMPs involves specific collagen integrin-receptors. The cell adhesion activity of the CMP1 was about 50% of that of collagen, verifying the GFOGER as the major binding locus of the type I collagen. The cell spreading morphology on the CMP1 was comparable to that observed on the collagen. The effect of the CMP1 on regulating cell morphology, proliferation and functions was examined by Hep3B cell culture on the CMP1-functionalized Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV) microspheres. The presence of the CMP1 matrix promoted cell attachment and spreading on the microspheres as well as extensive cell growth and cellular bridging as demonstrated by the total DNA content and thick tissue-like construct (thickness >100 μm). A three-dimensional distribution of CMP1 ligands on the microspheres improved albumin secretion and P-450 activity of the cells. Our results established the potential of the CMP1 to replace the native collagen in the matrix biology and tissue engineering applications. It can be used as a tool for specific cell-integrin targeting for optimizing cellular responses as well as improving cellular phenotypes and functions.

Introduction

Type I collagen is the most abundant protein in mammalians and the predominant component of ECM. It is prominent due to its unique triple helical conformation composed of three chains of repeating Gly-Xxx-Yyy sequences. Collagen serves as an important structural component to tissues and is intimately associated with cell proliferation, cell-cell and cell-ECM communications, migration and differentiation[1]. It is the primary resource for many biological applications [2]. However, the use of mammal-derived collagen, especially for implantation, is often restricted for the inherent problems associated with the handling of collagen. These include: the potential risk of disease transmission, insolubility in most physiological buffers, low purity, and poor reproducibility[1]. Thus, mimicking of some biological characteristics of collagen with various peptide sequences is essential to avoid these difficulties and to improve cellular responses in biomaterials. Several integrins, such as $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_{10}\beta_1$, and $\alpha_{11}\beta_1$, have been shown to bind the collagen and activate cytoplasmic intracellular signaling

pathways[14-19]. Cyanogen bromide (CB) fragments of the collagen α_1(I) chain have been used to identify cell recognition motifs within type I collagen. Previous studies revealed that GFOGER corresponding to residues 502-507 of collagen α_1 (I) is the major binding locus within the type I collagen [3,4]. Using transfected cells and recombinant I domain, the integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ have been shown to recognize the triple helical GFOGER and GFOGER-like motifs found in collagen[4]. To date, CMPs of comparable biological activity of the native collagen have not yet been extensively applied for tissue engineering. In this study, we have examined the cellular recognition and responses of our tailor-designed CMPs (Figure 1c) by Hep3B liver cells. The effect of a CMP on regulating cell morphology, proliferation and functions was also investigated. Our aim is to develop a collagen mimetic that can be used in biomedical and tissue engineering applications. Replacing the native collagen with a fully synthetic CMP may provide a versatile approach to designing new biomaterials, which are biologically safer, active, reproducible, and pure, for tissue engineering. This study presented an intriguing challenge toward realizing an artificial collagen.

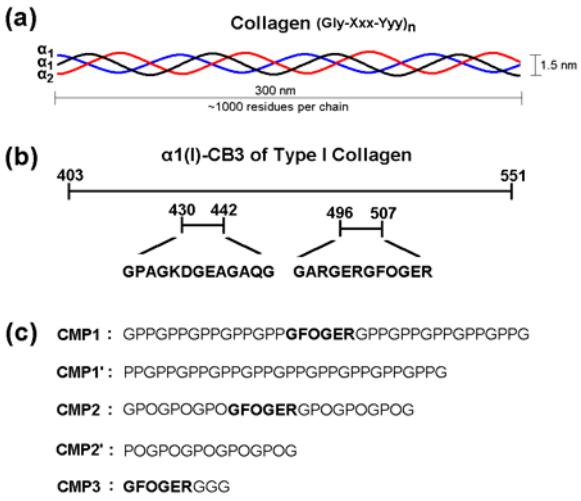

Figure 1. (a) Collagen triple helix. (b) Integrin-specific binding sites found on cyanogen bromide (CB) fragments of the collagen α_1(I). (c) Our tailor-designed CMPs.

Experimental

CMPs were synthesized using Fmoc solid phase peptide synthesis method and characterized by circular dichroism (CD) spectroscopy and melting curve analysis. The CMPs activity was studied by cell adhesion and competition inhibition assays. The effect of CMPs on regulating cell proliferation, albumin secretion, and P450 acitivity were studied by Hep3B cell culture on CMP1-functionalized PHBV microspheres.

Results and Discussion

Biophysical studies. The CMPs, except CMP3, exhibited CD spectra features characteristic of collagen-like triple helix, including a positive peak around 225 nm and a large negative trough near 200 nm (Figure 2). These CD spectra undergo a red shift in their band positions, as compared to that of the collagen, due to the higher percentage of imino acids contents. All CMPs, except CMP2' and CMP3, exhibited cooperative transition curves denoting the triple helix ↔ random coil transition. Thermal melting curve analysis verified that all these CMPs are stable in the form of triple helix at room temperature.

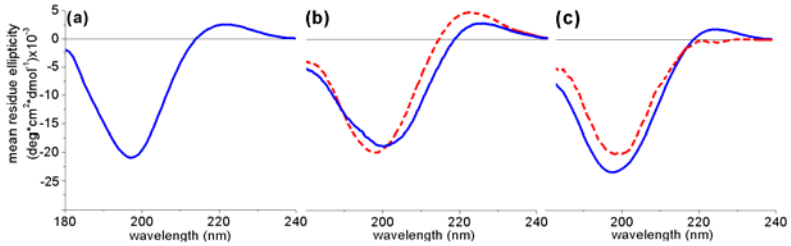

Figure 2. CD spectra of (a) collagen (0.50 mg/ml), (b) CMP1 (blue) (0.4 mg/ml)and CMP2 (red) (0.4 mg/ml), and (c) CMP1' (blue) (0.6 mg/ml) and CMP2' (red) (0.45 mg/ml) in water.

Cellular Recognition. The result of the cell adhesion assay (Figure 3) showed that the CMP1, CMP2 and collagen were well-recognized by the Hep3B liver cells and could support the cell adhesion in a conformation-dependent manner, with the CMP1 of most stable triple helical conformation being preferred. The result is in agreement with the fact that the native collagen has noticeably higher affinity for collagen specific receptors than the denatured one[5]. Similarly, absence of the GFOGER hexapeptide in the CMP1' and CMP2' caused an adverse effect on the level of adhesion of Hep3B cells (< 10%). The result verified that the GFOGER is the major binding site of the type I collagen. In this study, we reported that our CMP1 has ~50% of the adhesion activity of the native collagen. It is clearly visible that Hep3B cells spread extensively on the collagen and CMP1 (Figure 4). The Hep3B cell spreading was most efficient when the triple helices was combined with the collagen α_1(I) 502-507 sequence. The result given in Figure 3 showed that the GFOGER-containing triple helical CMPs effectively inhibited Hep3B cell binding to the collagen, verifying the cell adhesion to these CMPs is mediated specifically by a collagen integrin-receptor. The inhibitory activity of CMP1 was ~88% of that observed for the blank. Similar result was obtained for collagen.

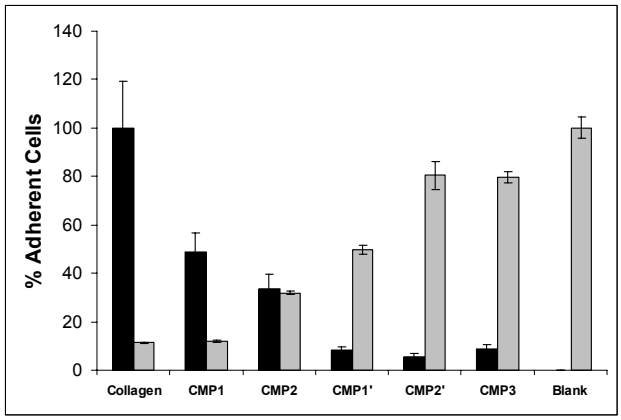

Figure 3. Adhesion of Hep3B cells on the sample (dark) at 1 hr in serum-free medium; and inhibition of cell adhesion on collagen by the samples (grey) at 1 hr in serum-free medium.

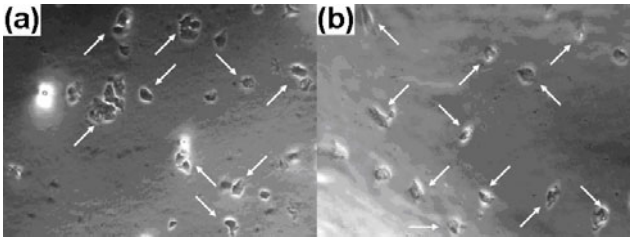

Figure 4. The cells spread well (as indicated by the arrow) on the (a) collagen and (b) CMP1.

Cell Proliferation and Functions. The Hep3B cells displayed significant improvement of proliferation when cultured on CMP1-functionalized (covalently) microspheres as compared to the blank control indicating that the ligands conjugation was successful and potent for making microspheres more conducive (Figure 5). The activity of the CMP1 in promoting Hep3B cell proliferation is identical to that of RGD, a well-known integrin-specific peptide for hepatocytes[6]. Albumin secretion is an important function displayed by liver cells. The result

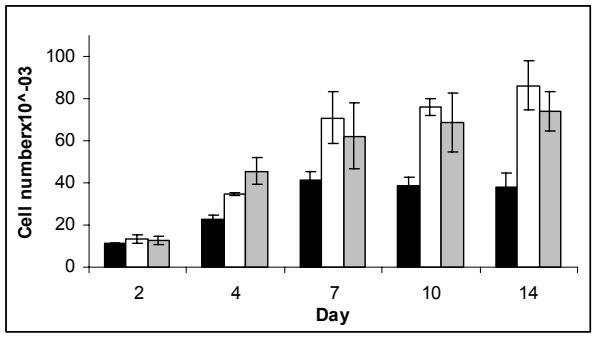

Figure 5. Cell proliferation of Hep3B cells on (a) blank (black), (b) RGD- (white), (c) CMP1- (grey) funtionalized microspheres over 14 day culture.

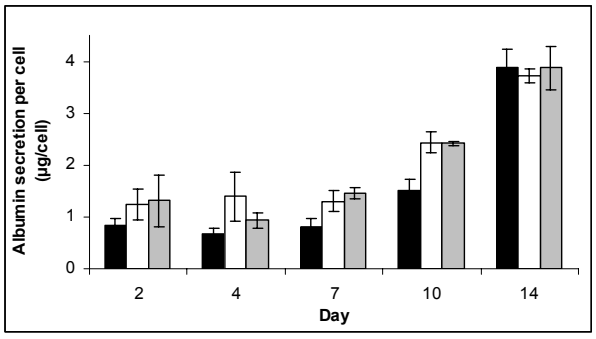

Figure 6. Albumin secretion by Hep3B cells on (a) blank (black), (b) RGD- (white), (c) CMP1- (grey) funtionalized microspheres over 14 day culture.

presented in Figure 6 is the albumin secretion on the cell number basis at different sampling periods. The cells cultured on RGD and CMP1-functionalized samples showed significantly improved albumin secretion functions, suggesting that the integrin-mediated cell adhesion, intra- and extra-cellular signal transmitting is essential for regulating central biological processes, including cell proliferation and functions. The result is in good agreement with the cell proliferation as there is often a tradeoff between the cell proliferation and differentiation[7]. The cells have grown to near confluent on the microspheres after 10 day culture and the proliferation rate slowed down thereafter and thus triggering an active differentiated function. The P-450 activity indicates the detoxification ability of single Hep3B cell over 4-hour sampling period on the test day. Generally, the cells exhibited a similar level of P-450 activities (result not shown). The cells showed much lower P-450 activities on day 14. This is possibly due to the restricted transport and diffusion of 7-ethoxyresorufin into the thick tissue-like structure formed around the microspheres of depth of more than 100 μm (Figure 7). The restriction reduced the contacts of the chemicals and the cells and could have resulted in a low P-450 activity. The thick multiple cell layers may also limit the exchange of nutrient, oxygen and waste and therefore vascularization would be a key step in making a successful liver tissue.

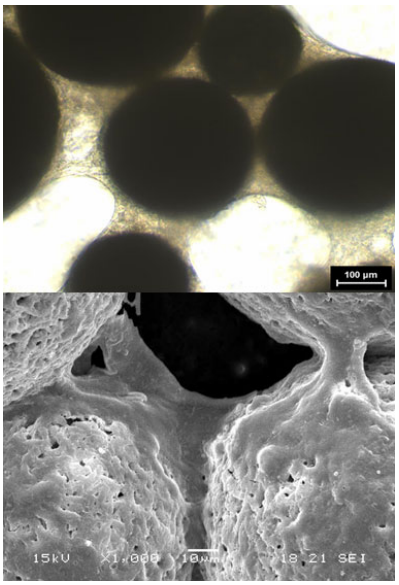

Figure 7. Light microscopy image of tissue-like construct (Top) and SEM image of multiple cellular layers (Bottom) grown on CMP1-functionalized microspheres after 3-day culture.

Conclusion

Mimicking of some biological characteristics of collagen using various peptide sequences presented an intriguing challenge toward realizing an artificial collagen. In this study, we demonstrated the efficacy of our CMP as cell and tissue support matrices and for use in tissue engineering to enable the *in vitro* growth of cells into large pieces of tissues. The generation of a biomimetic microenvironment using a fully synthetic, hence pure and reproducible, collagen analogue may provide a versatile approach to designing biologically active and safer matrix materials for biomedical and tissue engineering purposes.

Acknowledgement.

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