



Polyvalent display of RGD motifs on turnip yellow mosaic virus for enhanced stem cell adhesion and spreading

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ABSTRACT

Turnip yellow mosaic virus (TYMV) is a stable 28 nm icosahedral plant virus that can be isolated in gram quantities. In order to study the polyvalent effect of Arg-Gly-Asp (RGD) clustering on the response of bone marrow stem cells (BMSCs), an RGD motif was genetically displayed on the coat protein of the TYMV capsid. Composite films composed of either wild-type TYMV or TYMV-RGD44, in combination with poly(allylamine hydrochloride) (PAH), were fabricated by a layer-by-layer adsorption of virus and PAH. The deposition process was studied by quartz crystal microbalance, UV–visible spectroscopy and atomic force microscopy. BMSC adhesion assays showed enhanced cell adhesion and spreading on TYMV-RGD44 coated substrates compared to native TYMV. These results demonstrate the potential of TYMV as a viable scaffold for bioactive peptide display and cell culturing studies.

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1. Introduction

Mesenchymal stem cells (MSCs) represent an important alternative source of cells for tissue regeneration due to their ability to differentiate into various phenotypes, such as chondrocytes [1,2], skeletal muscle cells [3], osteoblasts [4,5], and vascular muscle cells [6]. MSCs reside in specialized niches within various tissues and can be isolated, cultured and expanded *in vitro* [7]. The differentiation of MSCs into various phenotypes can be triggered by the micro-environment around the cells [8]. To better understand how MSCs respond to environmental cues, a number of nanomaterials and a variety of nanofabrication methods have been employed to control the differentiation pathways of MSC [9–14]. Our previous studies have shown that culturing bone marrow stem cells (BMSCs) on plant viruses, including tobacco mosaic virus (TMV) and turnip yellow mosaic virus (TYMV), can enhance cell differentiation [15–17]. Enhanced osteocalcin expression and levels of osteo-differentiation were significantly augmented by the presence of virus coatings on two-dimensional substrate in comparison to cells grown under standard conditions (from 21–28 days to 9–14 days), resulting in earlier mineralization of the cells.

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Furthermore, we found that the differentiation was preceded by an upregulated expression of the bone morphogenetic protein-2 (BMP-2) gene, which encodes a key morphogenetic protein involved in bone formation, for cells cultured on native TMV within 8 h of osteo-induction [18]. However, plant virus-based substrates can often influence the differentiation of modest numbers of cells due to the lack of cell-binding motifs on the viral capsid.

Numerous studies have shown that the enhancement of the endothelial and osteoblast adhesion can be achieved by immobilizing adhesive peptides on the substrate surface [19–21]. The tripeptide motif RGD is often exploited for tissue regeneration applications due to its adhesion properties [21–26]. It has been reported that RGD-tailored bio-nanoparticles, produced either via genetic modification or chemical conjugation, can improve the cell-binding affinity dramatically [27–37]. For example, using a Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) reaction, synthetic RGD peptides have been conjugated to wild type TYMV, resulting in the promotion of adhesion, spreading, and proliferation of NIH-3T3 fibroblast cells [38]. We report here our work in replacing native coat protein (CP) residues within TYMV, through mutagenesis for display of RGD on the virus surface. Once assembled, this multivalent display of the desired RGD epitope enables the adhesion properties of BMSCs to be studied with predictive nanometer RGD-spacing.

TYMV is a non-enveloped 28 nm plant virus composed of 180 copies of a 20 kDa CP that encapsidates a 6.3 kb monopartite genome. The subunits assemble together with $T = 3$ symmetry that results in an icosahedral capsid with prominent protrusions of