Developing A Fully Synthetic, Peptide-Based, Animal-Free Model of Pancreatic Cancer

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Introduction

Pancreatic cancer (PC) is a lethal disease with high tumour tissue complexity, for the study of which human relevant models are needed. Similarly to many other human diseases, PC models are often built using animal-derived products to support cell attachment and growth. Despite offering good biocompatibility they lack accurate human tissue mimicry. In light of the recent Federal Drug Association (FDA) regulatory changes (1), efforts are made to reduce the use of animals and animal-derived products in drug screening as they fail to predict successful drug candidates. In this study we replace animal-derived protein coatings in our previously developed PC models(2–4), with synthetic peptides and assess their suitability for developing fully synthetic models of PC.

Aims & Objectives

To develop an animal-free model of PC using polyurethane and synthetic peptides

- Adapt PC cell lines to synthetic or human-derived sera
- Coat polyurethane (PU) with synthetic peptides (RGD, IKVAV) either via physisorption or plasma modification
- Assess peptide coating efficiency of physisorption and plasma modification
- Assess cell metabolic activity and viability in peptide-coated scaffolds

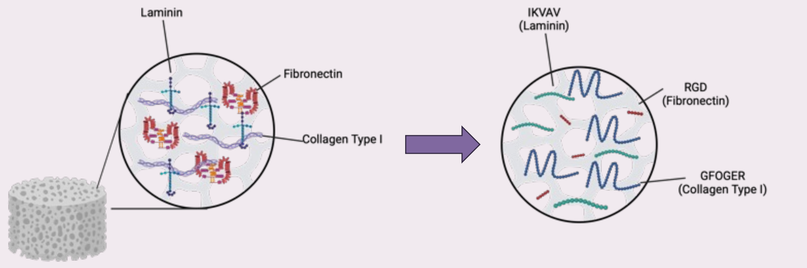


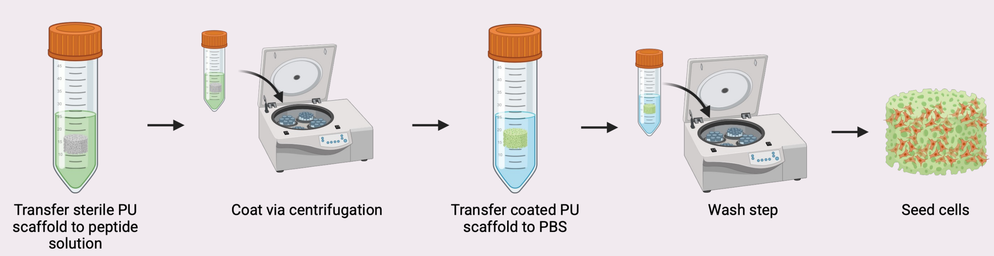
Figure 1. Replacement of animal-derived proteins with synthetic peptides. Polyurethane scaffold was historically decorated with full length animal-derived proteins (left) either alone or in combination of two. The transition to a fully synthetic scaffold involves replacing full length proteins with short peptide sequences namely IKVAV to replace laminin, RGD to replace fibronectin and GFOGER to replace collagen type I.

Methodology

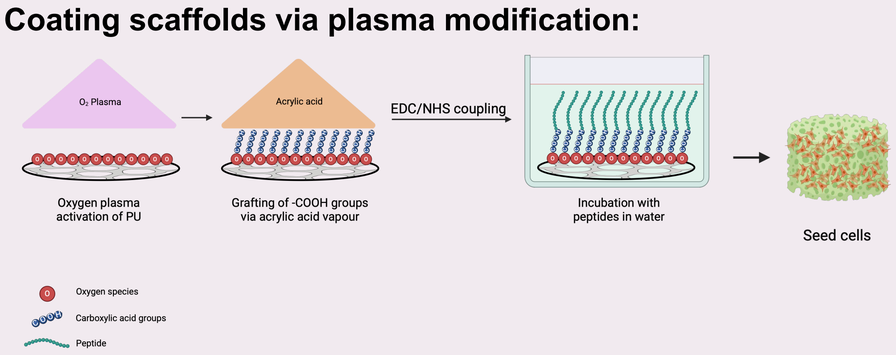
Cell culture conditions

PANC-1 cells frozen previously in FBS were thawed in DMEM containing 10% human-derived serum and adapted for two weeks before experiments. After that metabolic activity and cell proliferation were monitored for a period of 6 days. Adapted cells were subsequently used for downstream experiments.

Coating scaffolds via physisorption:



Coating scaffolds via plasma modification:



Results

1. PANC-1 cells grow efficiently in human-derived serum

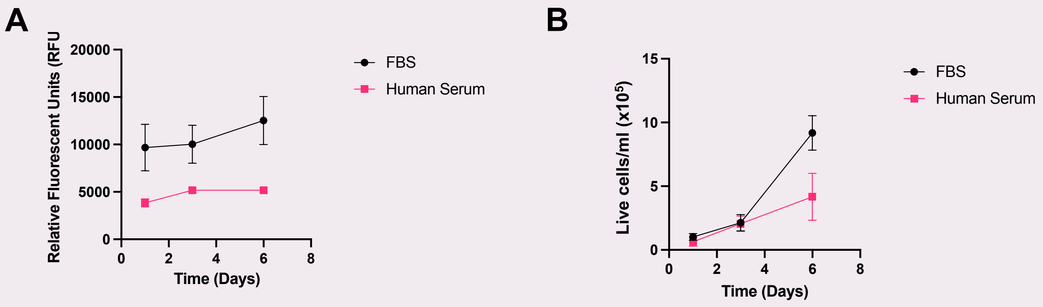


Figure 2. PANC-1 cells grown in human serum. A. Metabolic activity of PANC-1 cells transitioned to 10% human serum measured via alamarBlue 2 weeks after adaptation for 6 days. **B.** Cell proliferation of PANC-1 cells measured by trypan blue exclusion assay 2 weeks after adaptation for 6 days. All data compared to historic data of PANC-1 cells grown in FBS.

2. Synthetic peptides coat the PU scaffold uniformly via physisorption

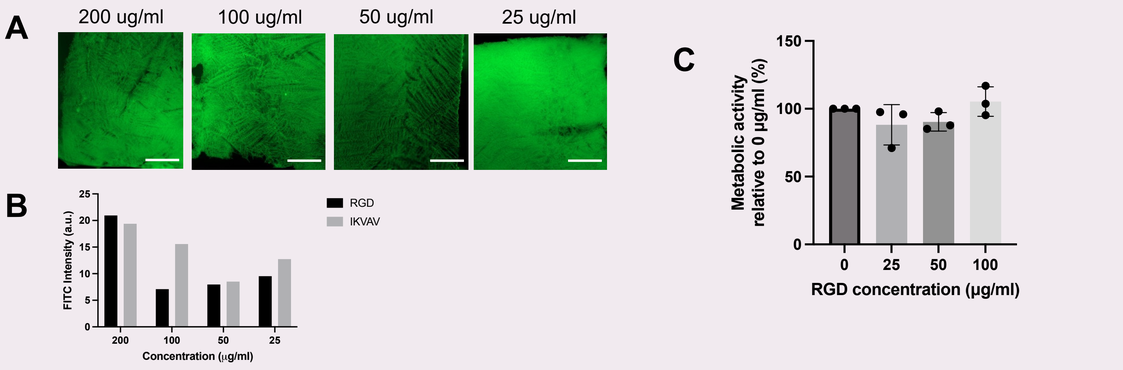


Figure 3. Physisorption peptide coating. A. Representative fluorescent images of PU scaffolds coated with FITC-tagged RGD peptide at different concentrations. Scale bar: 1 mm. **B.** FITC intensity measurements of FITC-tagged RGD and IKVAV peptides at different concentrations measured from different areas of sectioned PU scaffolds. **C.** Metabolic activity of PANC-1 cells seeded in PU scaffolds coated with varying concentrations of RGD peptide. Data shown relative to uncoated (0 µg/ml) scaffolds.

3. Plasma modification results in a uniform peptide coating with good biocompatibility even at low peptide concentrations

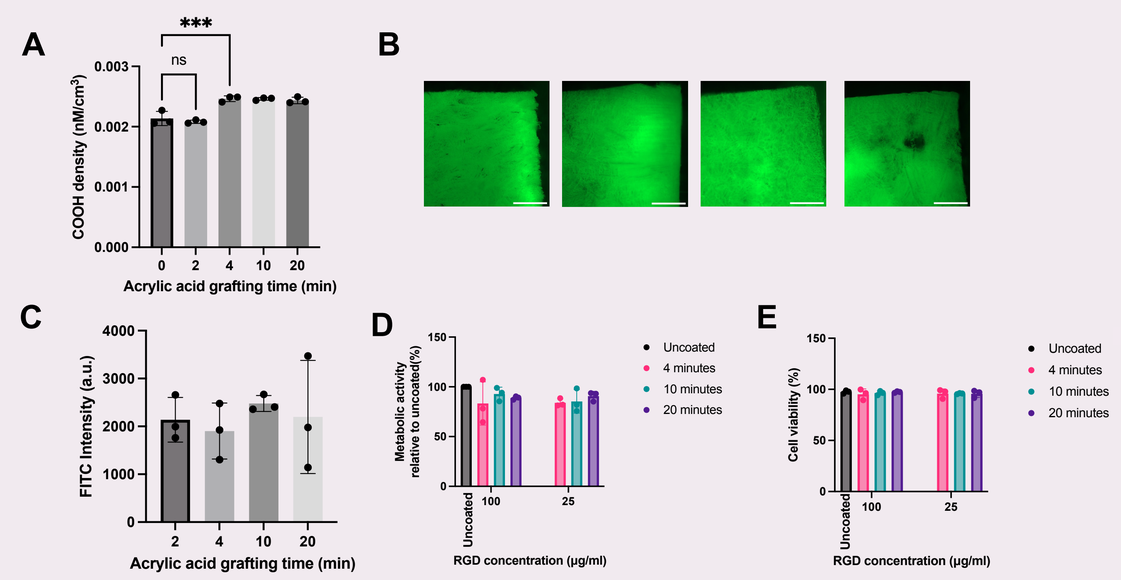


Figure 4. Plasma modification peptide coating. A. Measurement of COOH group density on PU scaffolds via the toluidine blue O assay. PU scaffolds were modified with plasma for 5 minutes and exposed to acrylic acid vapour for 2-20 minutes. **B.** Representative fluorescent images of sectioned PU scaffolds coated with RGD (100 µg/ml) following plasma modification with different exposure times to acrylic acid. Scale bars: 1 mm. **C.** FITC intensity measurements of FITC-tagged RGD peptide at different acrylic acid grafting times measured from different areas of sectioned PU scaffolds. **D.** Metabolic activity of PANC-1 cells seeded in plasma modified PU scaffolds for 21 days. **E.** Viability measurements of PANC-1 cells grown on plasma modified PU scaffolds coated with RGD peptides via LIVE/DEAD staining.

Conclusions & Future Directions

- PANC-1 cells can be easily adapted to human serum conditions
- Centrifugation and plasma modification result in uniform dispersion of peptide within the porous scaffold
- With plasma, peptides bind to the PU surface covalently resulting in a potentially longer lasting coating therefore requiring less material to be used
- Peptide coatings show good biocompatibility at all tested concentrations

References

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