Can Theoretical and Experimental Polymer-Polymer Miscibility Predictions Provide Insight to *in vivo* Compatibility in Caco-2 Cells?

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Background: Polymer-polymer miscibility refers to the ability of two polymers to mix in all proportions forming a homogenous phase. Knowledge of miscibility is important for tissue engineering applications – particularly in the generation of hybrid 3D printed polymeric scaffolds. Polymer-polymer miscibility can determine the complementary properties of a polymer composition. Immiscibility can result in a polymer composition being very brittle, reducing the printability of a polymeric scaffold and influencing the composition's ability to promote cell growth. Typical 3D printing scaffold fabrication methods include fused deposition modelling (FDM), and bioprinting. Hybrid scaffolds containing multiple polymers can be advantageous. For example, chitosan/polylactic acid (PLA) hybrid scaffolds have previously shown improved stiffness which is favourable for cartilage tissue engineering. This study aimed to identify miscible polymer combinations using theoretical and experimental approaches to inform polymer selection for scaffold development, and to identify the polymer combinations with optimal cell viability for the generation of FDM 3D printed polymeric scaffolds.

Methods: Polymer-polymer miscibility and cell viability of PLA, soluplus (SP), polyvinylpyrrolidone vinyl acetate (Kollidon VA64 (KV64)), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and their combinations were studied. To understand the nature of the cohesive forces between the polymers, theoretical polymer-polymer miscibility was determined by calculating the solubility parameters of the polymer combinations using the Van Krevelen and Hoftyzer, and Hoy methods. Experimental polymer-polymer miscibility and detection of hydrogen bonding were investigated using heat flux differential scanning calorimetry (DSC) (heating rate of 10ºC/min, between 0 ºC and 250 ºC) and attenuated total reflection- fourier infrared (ATR-FTIR) spectroscopy, respectively. The most miscible polymer combinations were used for cell viability studies in human colorectal adenocarcinoma (Caco-2) cells using the alamar blue cell viability assay and the resulting half-maximal inhibitory concentration (IC50) was determined *via* linear regression.

Results: Theoretical polymer-polymer miscibility calculations identified SP and PLA to be the most miscible polymers giving differences between solubility parameter ($\Delta\delta$) values of less than 10 $MPa^{1/2}$ when combined with all other polymers whilst PVA was least miscible. DSC experiments confirmed these results as SP and PLA combinations gave single glass transition (T_g) values in the range of 55°C - 66°C on cooling. PVA combinations largely gave multiple T_g's. ATR-FTIR studies of SP and PLA combinations showed little evidence of hydrogen bonding, with a slight broadening of the peak at 3500 cm^{-1} corresponding to interaction with the hydrogen bonding donors of SP. *In vitro* studies in Caco-2 cells of miscible combinations identified SP and PLA in a 70/30 *w/w%* combination to give the highest IC50 of 82mg/mL indicating the low inhibitory effect of the two materials. In many instances, Caco-2 cell viability improved greater than 100% when polymers were in combination with one another, e.g. SP and PVA in a 70/30 *w/w%* combination.

Conclusions: This study identified miscible polymer combinations using theoretical and experimental methods, which found SP and PLA combinations to be most miscible and certain PVA combinations to be least miscible. Cell viability studies of miscible combinations showed SP, PLA and PVA combinations to promote cell growth and enhance cell viability. These results identify potential polymer combinations for the generation of FDM 3D printed hybrid scaffolds for tissue engineering.

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Deleted: A pharmaceutical excipient is a substance other than the active pharmaceutical ingredient (API), which is present in a medicinal product or used in the manufacturing of the product. Many of the excipients used are synthetic polymers which function to enhance the products' stability, biopharmaceutical profile, appearance, patient acceptability, and processability. Currently, there is little research being carried out on the influence of excipients in pharmaceutical products – with researchers discovering excipients which were previously classified as in-active to in fact be active. Of particular interest, is understanding the influence of polymer-polymer miscibility in polymeric blends and their biological impact. Polymer-polymer miscibility is an important factor in obtaining the complementary effects of a polymer composition, and thus immiscibility can result in poor performance of a polymer blend. In this study, the polymer-polymer miscibility and *in vivo* toxicity of five polymers (polylactic acid (PLA), soluplus (SP), polyvinylpyrrolidone vinyl acetate (Kollidon VA64 (KV64)), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP)), widely used for drug delivery applications, was studied.

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