COLLEGE OF ENGINEERING
Department of
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Photo cross linkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering

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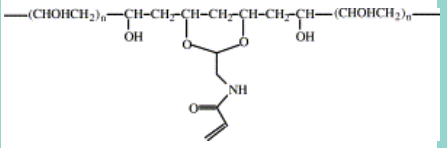
Abstract

Photoactive polyvinyl alcohol hydrogels (PVA) exhibit great potential as scaffolds for tissue engineering, providing in situ polymerization for implantation that requires minimal invasiveness. This study demonstrates the adaptability of PVA hydrogels for a range of soft tissue applications by investigating their mechanical characteristics and biological compatibility. Highly elastic hydrogels are produced by adjusting the crosslink able groups and polymer concentration to customize the tensile strength and Young's modulus. When fibroblasts are evenly placed within these hydrogels, they continue to produce extracellular matrix proteins and remain viable for two weeks. We functionalize naturally non-adhesive PVA hydrogels with the cell-adhesive peptide RGDS to improve cell adherence. Fibroblast adhesion and spreading are supported by this dose-dependent alteration. Under controlled circumstances, in situ formation is made possible by the quick and biocompatible photopolymerization process. Our study highlights the possibility of photoactive PVA hydrogels as adaptable scaffolds.

Methodology

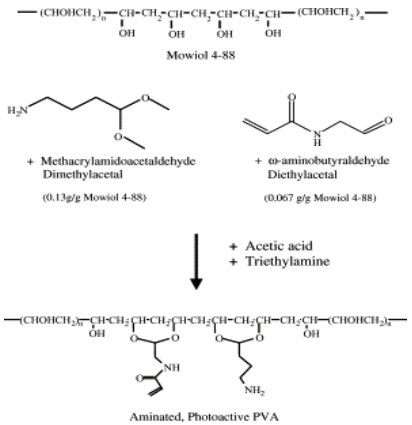
For photopolymerization, a 30-weight percent aqueous solution of polyvinyl alcohol (PVA) with an average molecular weight of 14,000 g/mol was used. Methacrylamidoacetaldehyde dimethyl acetal, was used to modify this PVA in order to add crosslinkable side groups (degree of modification=0.45 Meq/g). As the photoinitiator, Irgacure 2959 (0.1 vol%) was utilized. Hydrogels were created by crosslinking the polymer solution with long-wavelength UV light (365 nm, 10 mW/cm², Blak-Ray). It took 8 minutes to complete. To evaluate flexibility, more hydrogels with a 30-weight percent PVA solution (MW 37,000) and fewer cross linkable side groups (degree of modification = 0.19) were made. These hydrogels were meant to be tested mechanically. This process guarantees PVA hydrogels' quick and regulated photopolymerization, which is necessary for tissue engineering applications.



PVA's optimized chemical structure that is photoactive.

Methodology

Covalent modifications of polyvinyl alcohol (PVA) were made using RGDS (Arg–Gly–Asp–Ser) or RGES (Arg–Gly–Glu–Ser) peptides. The process of acetylating peptides involved lyophilization after a two-hour reaction with acetic anhydride (1:1 molar ratio) in HBS. Deionized water was used to dissolve acetylated peptides (0.5–5 mg/ml). The acetylated peptide was coupled by adding 2% N-ethyl morpholine and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) in a 2:1 molar ratio. The amine-modified PVA with crosslinkable groups was dissolved in DI water and then added at an 8:1 molar ratio (peptide:PVA) to the acetylated peptide/EDAC/N-ethyl morpholine mixture. After five hours of room temperature reaction, lyophilization and dialysis against DI water were performed. The polymer's amine groups' reactions to RGDS and RGES were evaluated using the ninhydrin assay. With the help of bioactive peptides, this technique ensures the effective covalent modification of PVA for improved cell attachment in tissue engineering applications.

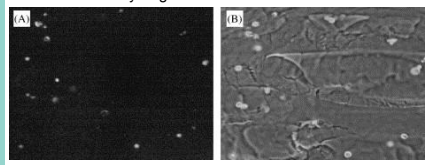


Aminated, photoactive PVA is created when PVA reacts with amino butyraldehyde diethyl acetal and methacrylamido acetaldehyde dimethyl acetal.

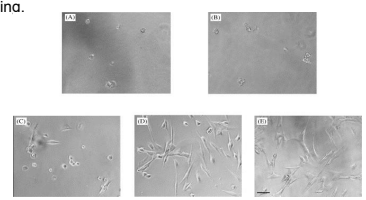
Statistical evaluation: Two-tailed, unpaired t-tests were used to compare the data sets. P values considered essential for those that were less than 0.05.

Results

Human Dermal Fibroblasts (HDFs) were successfully seeded homogeneously within 22.5 weight percent PVA hydrogels, and cell viability was evaluated at different intervals (0, 1, 2, 7, and 14 days). Surprisingly, during the two-week culture period, viable HDFs—which consistently surpassed 80%—were observed, indicating that cells survived the photopolymerization process. Crucially, at no point during the culture period were there visible variations in viability across the thickness of the hydrogel scaffold. These results highlight the potential of these materials for long-term tissue engineering applications by demonstrating the robust compatibility of HDFs with the photopolymerization process and their sustained viability within the PVA hydrogel.



The presence of fluorescent cells is demonstrated by CMFDA staining.



To investigate the bio specific adhesivity of added PVA hydrogels, the non-adhesive control peptide RGES was covalently bound to the cell adhesion peptide RGDS. These hydrogels were seeded with HDFs, and after 20 hours, the cells were observed. When HDFs were functionalized with tethered RGDS on PVA hydrogels, they showed strong attachment and spreading. On the other hand, many HDFs on unmodified PVA and PVA-RGES hydrogels were removed by rinsing, leaving the residual cells with a rounded shape. After 20 hours, protruding processes from HDFs attached to PVA-RGDS hydrogels (0.7, 1.4, and 2.8 mmol PVA-RGDS/ml) were visible under phase-contrast microscopy at 100x magnification. Measurements of cell area verified that attached HDFs on PVA-RGDS hydrogels spread more than on PVA and PVA-RGES surfaces. The spread cell area was found to be significantly impacted by the density of RGDS on the hydrogel surface. The findings highlight the potential of RGDS in tissue engineering applications by highlighting its importance in promoting adhesion of cells and spreading on PVA hydrogels.

Conclusion

Photopolymerizable PVA hydrogels show great potential as versatile scaffolds for tissue engineering applications. These hydrogels can be used in a variety of soft tissue contexts because of their adjustable mechanical qualities, which include ultimate tensile strength and Young's modulus. These hydrogels have the potential to be used for ex vivo or in situ cell encapsulation due to their uniform cell seeding, extended viability, and ability to be cultured in vitro. These materials' versatility in a range of applications, such as transdermal UV illumination, is further enhanced by their quick and effective photopolymerization process, which is compatible with minimally invasive procedures.

The study highlights the synthetic functionality of photoactive PVA hydrogels by showing how cells within them actively secrete matrix proteins like collagen. Furthermore, in a dose-dependent manner, the effective covalent binding of the cell adhesion peptide RGDS to hydrogels dramatically increases cell attachment and spreading. Because hydrogels can be modified with bioactive molecules, they become flexible platforms for influencing the behavior of cells.

PVA hydrogels have an edge compared to other photopolymerizable hydrogels, such as polyethylene glycol (PEG), and traditional materials like poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). PVA's tunable elastic properties, which can be adjusted by changing the crosslinkable groups, offer a special benefit for applications like post-implantation or bioreactors where mechanical stimuli transmission is necessary. PVA's pendant hydroxyl groups provide a multitude of attachment sites for bioactive molecules, such as growth factors and cell adhesion peptides. This adaptability permits the possible control of proliferation, migration, and differentiation in addition to enabling cell-selective adhesion.

PVA hydrogel scaffolds provide an exceptional combination of elastic properties and attachment sites for bioactive molecules, making them superior to current photopolymerizable hydrogels. Their versatility is further enhanced by the possibility of biodegradability, which makes them attractive options for a variety of tissue engineering applications. To advance the field of regenerative medicine, future research into the incorporation of multiple bioactive moieties aims to optimize the culture environment for diverse cell types.

Acknowledgements

Rachael H. Schmedlen, et al. “Photocrosslinkable Polyvinyl Alcohol Hydrogels That Can Be Modified with Cell Adhesion Peptides for Use in Tissue Engineering.” *Biomaterials*, Elsevier, 13 Aug. 2002, www.sciencedirect.com/science/article/pii/S0142961202001771.