Development of Tailored Bionanocomposites for Applications in Skin Tissue Regeneration

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Statement of Purpose: Tissue engineering is an interdisciplinary field that aims to develop synthetic substitutes that can replace, restore, maintain, and repair damaged tissue and eventually regenerate specific organs.[1] To this effect, several types of natural as well as synthetic materials have been developed, which include polymers, dendrimers, carbon nanotubes, collagen, gelatin and proteoglycans.[2] As the largest organ of the body and being highly exposed to the environment, skin plays a major role in the protection of the body's internal organs.[3] Thus far, most skin defects have been treated through the use of skin grafts, which are naturally derived from skin substitutes. The quandary with this approach is that there are not enough donors to supply the need of skin, and undesired immune response. It is therefore necessary to develop new biomaterials that can mimic skin tissue and can be engineered to regenerate skin. Appling the basic principles of tissue engineering in this work, we have utilized layer-by-layer assembly approach for preparation of biocomposites resulting from furan derivatives conjugated with short peptide sequences, followed by the incorporation of collagen. In addition to biocompatibility in the presence of dermal fibroblasts, we also tested the antioxidant ability of the materials to prevent free radical damage. Such biocomposites may have potential applications in skin tissue regeneration.

Methods: Synthesis of the biomaterials were carried out as follows. First, the base scaffold material was synthesized by conjugating the short peptide sequence Gly-His (1.5 mM) with furan-2-carboxylic acid (3-aminopropyl) amide (1.7 mM) by traditional peptide coupling methods[4] using coupling agents such as N-hydroxy succinimide and *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride. The solvent utilized was DMF. Upon completion of the reaction, the solvent was rotary evaporated and the product was recrystallized from methanol. The formation of the product was confirmed by FTIR (Thermo Scientific, Nicolet IS50) and ^{1}H NMR (300 Mhz , Bruker) spectroscopy. The formed product was then allowed to self-assemble under aqueous conditions for a period of seven to ten days. The self-assembled nanoassemblies were then allowed to conjugate with Type-I collagen in order to mimic the components of skin tissue. In addition, peptide sequences specifically implicated in wound healing were then incorporated. Finally, growth factors such as epidermal growth factor (EGF) were added to encourage cell differentiation and proliferation. All starting materials were purchased from Sigma Aldrich or from Fisher Scientific (for cell studies). The morphologies of the assemblies and the corresponding incorporation of each layer was confirmed by SEM (Zeiss-EVO 10) microscopy and FTIR spectroscopy.

To examine the effect of the nanoscaffolds on human dermal fibroblasts (MDF), cell proliferation assays were conducted over a period of 24-126 hours. Briefly, MDF cells were plated in culture media in 24-well plates at a density of 4×10^4 cells per well. After allowing the cells to attach and spread, biocomposites of varying quantities (20 μL to 100 μL) were added into wells containing 1mL final volume of media and incubated with the cells over time. To determine cell viability, trypan blue exclusion was carried out after varying periods of time. Antioxidant studies on the biomaterials were carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the absorbance of at 510 nm was monitored.

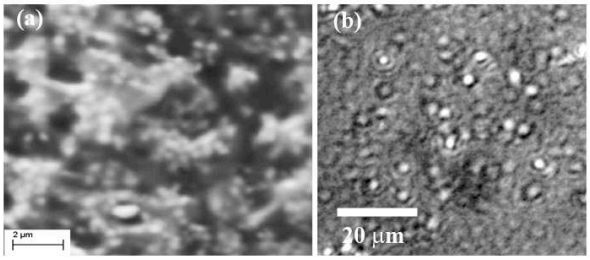

Figure 1. (a) SEM image of biocomposite; (b) Optical microscopy image showing attachment of biocomposites to fibroblasts after 48 hours of incubation.

Results: The biocomposites formed by layer-by-layer assembly on to the furan derivative conjugated with Gly-His displayed mesh like structures with diameters in the range of 500 nm to 1μ as indicated by the SEM (Figure 1a). H-bonding interactions between the peptide components as well as stacking interactions between imidazole and the furan moieties promoted the formation of the assemblies. Upon conjugation with collagen and wound healing peptide sequences distinct changes in morphologies were observed confirming their integration. This was also confirmed by FTIR spectroscopy with shifts in the amide I and II regions due to the incorporation of each layer. Cell studies in the presence of dermal fibroblasts showed attachment and proliferation under optical microscopy (Figure 1b) while trypan blue studies showed that 92% of the cells were alive after 126 hours.

Conclusions: A new biocomposite derived by conjugating short peptides with furan derivatives was prepared by the layer-by-layer assembly. The biocomposite exhibited biocompatibility and may have potential applications in skin tissue regeneration.

References:

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