HEPARIN AND HEPARAN SULFATE HYDROGELS FOR CARDIOVASCULAR TISSUE REGENERATION

Aliza Janse van Rensburg[a], Anel Oosthuysen[a], Linda Callanan[b], Neil Davies[a], Peter Zilla[a], Deon Bezuidenhout[a]

[a]Cardiovascular Research Unit, Chris Barnard Building, Anzio Road, Observatory, 7925, jvr.aliza@gmail.com
[b]Department of Process Engineering, Stellenbosch University, Banghoek Road, Stellenbosch, 7600

ABSTRACT

Heparin (Hep) and heparan sulfate (HS) have been shown to possess anti-coagulative properties, inhibit smooth muscle cell proliferation, moderate inflammation and control angiogenesis by potentiation of growth factors (GF). These properties are potentially very useful for the treatment of cardiovascular diseases, especially when delivered as hydrogels that form in situ. This project focuses on developing Hep and HS hydrogels for application in vascular grafts and myocardial infarction treatment (MIT).

Hep and HS were acrylated according to the Schotten-Baumann reaction and the products evaluated using Nuclear Magnetic Resonance (NMR) spectroscopy. The acrylated materials were cross-linked by a reaction with PEG multi-thiols (10PEG-4SH; Ac:SH = 1:1), and resulting gels were incubated in phosphate buffered saline (PBS) at 37°C. Hydrogel swelling, Hep/HS elution (3-methyl-2-benzothiazoninone hydrazine, or MBTH, assays) and Hep/HS activity (thromboelastography, TEG) were followed over an extended period (72days) in vitro.

Vinylic proton NMR peaks between 6-7 ppm confirmed the successful acrylation (40% and 80%; based on dimers). Gels formed after 5-6 minutes at physiological conditions (37°C, PBS, pH 7.4) with simple add-mixture of the acrylated Hep/HS and 10PEG-4SH solutions (10% nominal Hep/HS concentration). After rapid initial swelling (1-2 days) the gel mass increased steadily until disintegration at approximately 72 days. Similarly, after the initial burst release of Hep/HS (1-2 days) gels showed near linear (zero order) cumulative Hep/HS release over the remaining period ($R^2 \geq 0.98$). Addition of HS eluates to whole blood in the TEG assay resulted in delayed clotting, but the effect was not as pronounced as that achieved with Hep, either in the original form, after chemical modification, as well as after the incorporation and release from the hydrogels.

The degree of acrylation could be controlled by manipulating the reaction conditions and gels could be formed by Michael-type nucleophilic addition chemistry without additional initiators or by-products. Initial swelling was due to equilibrium water uptake followed by continued swelling due to hydrolytically degradation of the cross-linked network. The initial Hep/HS burst release can be ascribed to incomplete covalent incorporation combined with rapid initial equilibrium swelling. The zero order release obtained after covalent incorporation of Hep and HS in the gels is usually unattainable by simple incorporation and diffusion control release mechanisms. HS is known to be less anti-thrombotic than Hep; this property is retained and potentially very useful where GF binding without strong anticoagulation is desired.

These heparin gels are potentially very useful for vascular graft applications where their potent anticoagulative properties could help retain patency while the GF binding and anti-inflammatory properties facilitate angiogenesis and healing. For cases where strong anti-coagulation is not desired (such as MIT), but angiogenesis and anti-inflammatory properties are, analogous heparan sulfate gels have been developed.

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