Biocompatibility Evaluation of Electrospun Collagen, Gelatin, Polycaprolactone and their Composite Matrices in Rattus Norvegicus

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Abstract

The present study records on in vivo evaluation of five types of electrospun nanofibrous matrices, viz., collagen, gelatin, polycaprolactone (PCL), collagen-PCL (1:1) and Gelatin-PCL (1:1), prepared with a novel benign solvent in a rat model on wound healing. Granulation tissue formation was observed in the subcutaneous implanted tissue. There was no indication of gross rejection of the implants. The implantation did not result in major necrosis or extensive wound formation at the implantation site. The healing of the sutured incisions was also rapid with no scar formation after seven days of implantation. This study confirms that the five electrospun matrices were biocompatible and collagen-PCL biomaterial was found superior.

Key words: Electrospinning, Biocompatibility, Nanofibrous matrices.

Electrospun nanofibrous matrices are widely used since these mimic native extracellular matrix which enhances cell adhesion, cell signalling and cell-matrix interaction resembling the in vivo microenvironment and can be used as a tissue replacement to provide support for the cells. A novel solvent was used for electrospinning using collagen, gelatin and PCL.

Materials and Methods

Fish collagen type I (8% w/v), 19% (w/v) gelatin and 12% (w/v) PCL each were separately prepared as a homogenous polymer solution in acetic acid and DMSO (93:7) to obtain nanofibrous matrices. Composite nanofibrous matrices were prepared by mixing the dissolved polymer of PCL with collagen and gelatin in equal ratio.

The homogenous polymer solution was supplied through needle with a constant flow rate using a peristaltic pump. A high voltage power supply was applied to the needle and the collector to develop an electric field. Cross-linked collagen, gelatin and their composites (Michael et al., 2010) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and sterilized in graded and absolute ethanol, washed in PBS and stored at 4°C in PBS with antibiotics. Six male Sprague Dawley rats (250-275g) were used (IAEC/XXII/SRU/174/2011). A rectangular 1x3 cm full thickness surgical skin wound was created on the upper back to implant nanofibrous matrices and sutured. After 7 days, sutured area was excised, fixed in 10% neutral buffered formalin and processed paraffin sections were stained with haematoxylin and eosin. Inflammatory responses were graded (Subramanian et al., 2013; Table I) and quantified as very minimal, minimum, mild, and moderate.

Results and Discussion

The best result was obtained in the matrix development by dissolving the matrix material in acetic acid and DMSO in the ratio 93:7 and when the voltage was 16.5 KV, the distance of 23 cm and a flow rate of 0.6 rpm/min. Scanning electron microscopy of 5 different nanofibrous matrices showed the retention of their structural stability and integrity till 60 days of storage. There were no morphological swelling of fibers with retention of the initial crosslinking and the fibers thickness. The fibers appeared smooth without beads and were porous showing excellent interconnectivity mimicking the native extracellular matrix (Oraby et al., 2013; Zhan and Lan, 2012).

Collagen being the natural component of...
the biological system and the denatured product gelatin has been widely tested for biomaterials. Polycaprolactone being synthetic and their degraded products have been confirmed to be non-toxic products. These biodegradable polymers were chosen to electrospin using glacial acetic acid and DMSO solvent as benign combination to overcome the toxicity effects of the organic solvents to fiber integrity and the environment commonly used for electrospinning. Especially in case of collagen electrospinning which maintains its ultrastructural integrity of triple helical structure confirmed by transmission electron microscopy showing the 67 nm banding pattern (Fig. 1) of D periodicity (Burck et al., 2013; Zeugolis et al., 2008) which has been considered as fingerprint of fibrous collagen. This novel combination of solvent favours polycaprolactone to electrospin fibers with a wide range of concentration from 10% to 15% weight/volume to obtain fibers (Juliana et al., 2013; Schueren et al., 2011) from nanorange to microrange as the concentration increases (Elamparithi et al., 2015) can potentially modulate the elasticity for organ specific either hard or soft tissue engineering applications. This polycaprolactone solution was able to blend with collagen and gelatin which can further desirably tuneable to obtain tissue specific matrix modulus for tissue engineering applications.

In *in vivo* testing, there were no apparent changes in the behaviour, health status or the feeding activity of the animals. The gross wound healing as observed daily for the study period of seven days showed good progress. Histopathology: The inflammatory markers as observed for each of the study animals are presented in Table II. Biocompatibility evaluation showed moderate increase in polymorphonuclear cells, giant cells, lymphocytes, macrophages and plasma cells. This has been found to be normal at the early time point of a foreign body reaction. Granulation tissue formation grade response showed positive sign for the nanofibrous matrices. Collagen and composite of PCL and gelatin showed excellent granulation tissue formation whereas moderate granulation tissue formation to gelatin, PCL and composite of PCL and collagen. The nanofibrous matrices of gelatin and composite of PCL and gelatin showed necrotic areas with neutrophils. PCL showed scab formation along with lymphocytes, neutrophils, plasma cells.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell Number</th>
<th>Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>0-3</td>
<td>+</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>4-6</td>
<td>++</td>
</tr>
<tr>
<td>Giant cells</td>
<td>7-9</td>
<td>+++</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>10-12</td>
<td>++++</td>
</tr>
</tbody>
</table>

Quantification of the inflammatory cells present in the tissue section of implants at 7-day time point. (+ indicates very minimal, ++ minimum, ++++ mild, ++++ moderate tissue response)

<table>
<thead>
<tr>
<th>Cell types</th>
<th>SHAM control</th>
<th>Collagen</th>
<th>Gelatin</th>
<th>PCL</th>
<th>Collagen+ PCL</th>
<th>Gelatin+ PCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Giant cells</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Granulation tissue formation</td>
<td>-</td>
<td>excellent</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>excellent</td>
</tr>
<tr>
<td>Tissue response</td>
<td>-</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Table I. Average number of inflammatory cells in 40x magnification from at least 10 fields

Table II. Cell response to implants at least from 10 fields in 40x magnification
macrophages and giant cells with granulation tissue formation around the implant. There were no inflammatory cells observed in SHAM control. Minimal fibrosis in collagen and fibrous encapsulation around nanofibrous matrix in composite of collagen and PCL were observed (Fig. 2). Collagen and its composite showed lesser inflammatory cells when compared with the other three nanofibrous matrix implants which confirms that the healing process (Anderson, 2001) is faster in collagen and its composite when compared with gelatin, PCL and its composite.

Summary

The smart nanofibrous matrices developed with natural and synthetic bio-degradable polymers mimicked the in vivo extracellular matrix and collagen based matrices are more ideal to create artificial tissue constructs.

Acknowledgement

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References


