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In vivo biocompatibility of fabricated polymer implant in buffaloes - a preliminary study

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To address anestrum, different hormonal preparations were used to stimulate the hypothalamo-pituitary-ovarian axis for resumption of cyclicity in buffaloes (Das and Khan 2010) with varied results. Usage of these hormonal preparations revealed that progesterone based treatment regimens were more effective in inducing ovarian activity in anoestrus buffaloes (Wiltbank *et al.* 2011). Use of Crestar resulted in higher estrus induction rate (>80%) with conception rate of 45–60% both in cattle and buffaloes in comparison with other progesterone release device to mimic the luteal function and cause ovarian rebound on withdrawal for initiating ovarian cyclicity (Malik 2005, Wiltbank *et al.* 2011). These releasing devices use different polymers for sustained drug delivery, which can either be hydrophilic or non-hydrophilic. Studies showed use of polymers based on the necessity, chemical properties and compatibility *in vivo* (Chen 2010, van Werven *et al.* 2013). Testing of polymer for bio- compatibility is imperative for their subsequent usage for drug delivery *in vivo* and such studies have not been attempted in buffaloes. In this context, the present study was designed to test *in vivo* bio-compatibility of fabricated polymer implant in buffaloes.

Murrah buffaloes (7), multiparious buffaloes aged between 4.5–6.5 yrs with body weight ranging between 400–550 kg (BCS>3), maintained at the institute were managed on semi-intensive system and fed with *ad lib.* green fodder, wheat straw (2–2.5 kg), concentrate feed, mineral mixture daily. All the experimental procedures were carried with the approval of the Institutional Animal Ethics Committee.

In this study, non-biodegradable polymer, viz. polydimethylsiloxane-co-ethylhydrosiloxane was used for implant preparation. Implants were prepared according to Kit's protocol with modifications as non-biodegradable polymer, viz. polydimethylsiloxane-co-ethylhydrosiloxane (0.75 ml) along with curing agent was used for implant fabrication. The polymer and curing agent were mixed thoroughly and the resultant mixture was completely out-gassed and cast into desired implant molds. The poured mixture was cured at room temperature overnight and the solidified implants were cut out of the molds. The standardized dimensions of the fabricated cylindrical implants were 2.5 cm long and 0.3 cm diameter and stored at room temperature (20° C) till further animal trials. *In vivo* study was carried out to deduce the bio-compatibility of polymer implant on the physiological parameters of the animals. Fabricated polymer implants were inserted as ear implants in 7 buffaloes taken for this study. Local tissue reaction as well as systemic response, determined from serum biochemical parameters was carried out. For this, blood sample was collected from implanted buffaloes on day 0, 7 and 15 of the study. On day 15th of the trial, the implants were removed, biochemical analysis was done in automatic biochemistry analyzer using commercial kits. The data were analyzed statistically as per standard (Snedecor and Cochran 1989) using SAS software. All parameters were analyzed using paired *t*-test. All results shown were regarded significant at $P<0.05$.

In vivo compatibility and tissue reaction post implant insertion is shown in Fig.1. Biochemical parameters obtained from start (day 0) till removal of implant (day 15) are given in Tables 1 and 2, respectively. *In vivo* testing of the polymer was done for the first time in buffaloes. The polymer has been widely used in varied biomedical applications due to their physiological inertness, high blood compatibility, low toxicity, good thermal and oxidative stability, and low modulus in human biomedical devices (Chen 2010). This was evident from the present study showing no local and tissue reaction from day 0 to day 15 of study (Fig.1). This was in consonance with earlier report (Hassler *et al.* 2011) where limited inflammatory reaction was observed in polymer implant study *in vivo.* Moreover, in this study at the end of the trial, retrieval of the implants confirmed the non-biodegradable property of these implants as reported earlier (Hassler *et al.* 2011).

Furthermore to its low tissue reactivity, no systemic reaction was confirmed which was evident by no change in biochemical parameters corroborating its chemical inertness and non-toxic properties which make it a potential candidate for usage of drug delivery devices (Table 1). Studies showed

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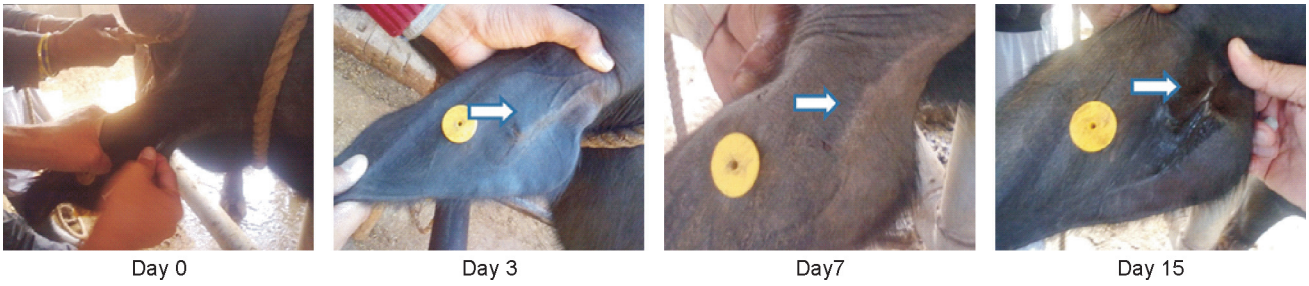

Fig. 1. Post implant local tissue reaction.

Table 1. Biochemical parameters during *in vivo* biocompatibility study

Parameter(units)	Day 0(N, 7)	Day 7(N, 7)	Day 15(N, 7)
LDH (U/l)	1192.47± 115.16[a]	1085.59± 88.39[a]	1013.36± 61.40[a]
SGOT (U/l)	96.77±12.73[a]	93.05±10.47[a]	93.45±10.97[a]
SGPT (U/l)	41.47±3.35[a]	41.77±3.23[a]	47.81±4.43[a]
ACP (U/l)	0.5±0.17[a]	0.21±0.04[a]	0.50±0.14[a]
CKNAC (U/l)	50.6±3.20[a]	60.56±8.16[a]	51.80±4.10[a]
Creatinine (mg/dl)	1.49±0.16[a]	1.59±0.16[a]	1.34±0.06[a]
Urea (mg/dl)	81.45±4.37[a]	85.87±4.12[a]	76.10±4.02[a]
Cholesterol (mg/dl)	126.44±4.48[a]	120.07±2.65[a]	117.35±2.59[a]
Triglyceride (mg/dl)	32.67±0.35[a]	30.55±0.60[a]	32.80±0.79[a]
Uric acid (mg/dl)	2.49±0.17[a]	2.21±0.10[a]	2.98±0.25[a]
Total protein (g/dl)	8.98±0.13[a]	8.61±0.05[a]	8.52±0.03[a]
Total bilirubin (mg/dl)	0.08±0.01[a]	0.05±0.01[a]	0.12±0.03[a]
Albumin (g/dl)	2.97±0.02[a]	2.88±0.04[a]	2.94±0.04[a]
Chloride (mmol/l)	100.7±0.74[a]	106.51±1.95[a]	104.11±0.26[a]

Values in a row with different superscript differ significantly; (P>0.05; mean±SE).

that polymorphonuclear leukocytes (PMN) can be activated by pathogens and artificial materials. The increase in their number indirectly indicated triggering of inflammatory reaction through reactive oxygen species, enzymes and pro-inflammatory cytokines. Activation of PMN, in particular, involves alterations in cluster of differentiation molecules and selecting leading to enhanced adhesiveness and initiation of degranulation of PMN cells (Nag and Banerjee 2012). This is important as activation of inflammatory reaction shall damage both implanted biomaterials and surrounding tissues as well. Reports showed that polydimethylsiloxane-co-ethylhydrosiloxane possesses unique properties of gas permeability, transparency and flexibility along with its inertness, non-toxicity and bio-compatibility (Hassler *et al.* 2011, Nag and Banerjee 2012). Earlier report showed that increase in serum enzymes, viz. LDH, ACP, CKNAC along with SGOT, SGPT was observed as indicators of liver and tissue damage (Hammam *et al.* 2011). Therefore, from the present study, polydimethyl-siloxane-co-ethylhydrosiloxane polymer was successfully used *in vivo* trials for testing bio-compatibility in buffaloes.

Solid matrix polydimethylsiloxane-co-ethylhydrosil-oxane implants were successfully used for *in vivo* trials in buffaloes. In addition, these polymer implants showed no local and systemic reaction evidenced by insignificant changes in serum biochemical parameters, ascertaining non-toxicity, low tissue reactivity, non-biodegradability and *in vivo* bio-compatibility of the polymer implants.

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