Transglutaminase crosslinked gelatin as an adhesive cell delivery system for tissue repair

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INTRODUCTION:
Tissue damage or degeneration such as rotator cuff tears and intervertebral disc degeneration are common problems that occur as people progress with age. A potential therapy to enhance healing of such problems is to deliver isolated mesenchymal stem cells (MSCs) to the site of the lesion. A key issue within this technology is the development of an injectable cell delivery system which is biocompatible, biodegradable and adhesive to the wound site. The objective of this study was to explore the possibility of using gelatin gel crosslinked with transglutaminase (Tg) as a delivery system for MSCs.

METHODS:
Gelatin/Tg gel preparation: Microbial Transglutaminase (Tg) from Streptomyces mobaraense was obtained from Ajinomoto and further purified with SP Sepharose fast flow column [1]. To prepare the crosslinked gel, Tg concentration was 25µg/ml in 10% gelatin (bovine skin of 225 bloom, Sigma). Gel mixture was incubated at 37°C 1 hour before in vitro and in vivo studies.

Burst test: A burst test was conducted to determine the endurance of the gel/Tg binding to the collagen matrix. Fresh bovine pericardium disc (4cm diameter) was used in vitro to as a collagen matrix and mounted on a water column. A 4mm diameter hole was punched in the center of the pericardium and filled with 250µl of freshly mixed gel/Tg. After 30min of gel curing time, constant water pressure with 735pa was applied. Burst time was recorded. Test was performed in a 37°C environment.

Cytotoxicity and cell migration on gel/Tg film: Cell directly overlaid on gel/Tg was conducted to test cell attachment and proliferation. In each 24 well plate, 200µl/well of gel/Tg gel was loaded and air dried. After rinsing with PBS twice, 50k/well of rat bone marrow stromal cells (MSC) [2] were seeded in 1 ml of 10% FBS/DMEM (Mediatech). Cell numbers was counted following trypsin detachment at 72 hours. Cell attachment and migration was observed on a daily basis.

Biocompatibility of Gel/Tg: Rat subcutaneous injections were conducted to test the gel/Tg biocompatibility. At the dorsum site, 1cc of the gel/Tg was injected through a 23 gauge needle at four sites for each Fisher 344 rat. Samples were retrieved after 2 and 4 weeks post surgery followed by histological analysis (n=4). All animal protocols were approved by USC IACUC.

Cell delivery test: Gel composed of 10% gelatin, 10X PBS, and Tg in a ratio of 9:10:0.025 was gently mixed with 1x10^6/ml of MSC cells and 40 µl of the mixture was injected (23G needle) into each well of 96 well plate. After 30min, 200µl of 10% FBS/DMEM (Mediatech) was added to cover the gel and medium was changed every 2-3 days. Cell viability was tested after 3 days by trypan blue staining and cell morphology was monitored up to 10 days.

RESULTS:
The gel/Tg did not dissolve in PBS at 37°C and maintained its shape for 2 weeks whereas gelatin dissolved in 24 hrs at 37°C.

Gel/Tg sealed defect stood water pressures of 735Pa for 15mins at 37°C. However, gelatin control burst immediately when water pressure increased beyond 245Pa.

Gel/Tg was not cytotoxic to the cell when tested with direct overlay models. After 48hrs from seeding, the cell numbers between the gel/Tg coated surface and the plastic surface had no significant difference [Fig. 1A]. MSC attached and proliferated equally well on gel/Tg coated section of the plate and plastic surface (48 hrs). [Fig. 1B]

After 2 weeks of implantation in rat, gelatin/Tg showed little or no inflammatory response with low numbers of neutrophils and no signs of necrosis. [Fig 2A] Gel/Tg was retrievable after 4w implantation but a significant decrease in gel size was observed. Fibroblasts were infiltrated into the gel to remodel the matrix. [Fig 2B]

FIG. 1:

Fig 1: Cytotoxicity test. A. Cell counts on gel/Tg and plastic surfaces. B. Cell attachment on plastic and gel/Tg surfaces.

Fig 2: H&E staining on gel/Tg implants A. 2week (40X) and B. 4 weeks (100X)

When gel/Tg was tested as cell delivery vehicle, encapsulated cells exhibited round shapes in the first 72 hours and cell were viable (trypan blue stain). Colonies were formed after 72 hours [Fig 3A]. By the 9th day, cells developed a network-like structure [Fig. 3B]. Gels seemed to be degraded by cell proteases and transparent digested rings were noticed in the gel (data not shown).

FIG. 3:

Fig 3: Gel/Tg as cell delivery vehicle tested in vitro. A. MSC colony formations at 3 days. B. Network like structures formed by MSC cells at 9 days.

DISCUSSION:
In this study, we have developed a transglutaminase crosslinked gelatin gel for minimum invasive cell delivery. We have shown that the gel/Tg is a non-toxic, adhesive and biodegradable hydrogel and has the potential to carry cells to a wound site. When homogeneously mixed with cells, gel/Tg composite can be locally delivered to the lesion site by injection. The gel/Tg not only maintains the carried cells to be viable and proliferative, but it also serves as a scaffold to support the migration of surrounding cells for tissue repair. The in-situ cross-linking between gelatin chains and endogenous collagen of tissue ECM can create a strong attachment between the matrices and prevent cells from diffusion. The potential of using this cell delivery system for rotator cuff tear and intervertebral disc repair are under investigation.

REFERENCES: