Development of a Self-Expanding Patch for Fetoscopic Myelomeningocele Repair

Rigwed Tatu[1], Marc Oria[2], Jose L. Peiro[2], Chia-Ying Lin[1].
[1]University of Cincinnati, [2]Cincinnati Children's Hospital Medical Center.

Statement of Purpose: Myelomeningocele (MMC) is the most harmful type of spina bifida, a permanently disabling defect affecting 1-2 neonates per 1000 live births; where the spinal cord elements are exposed, creating risks of contact with amniotic fluid and cerebrospinal fluid leak [1]. The latest repair technique involves fetoscopic patch coverage through small trocar ports [2]. Currently used patches need advanced endoscopic surgical skills for positioning at surgical site, and inert patches necessitate postnatal removal surgeries, which lead to monetary burden and psychological trauma [3,4]. Extracellular matrix-based patches or biological scaffolds lead to high production costs, and lead to loss of strength before transfer of functions to new host tissue [3,5,6]. The solution can be biodegradable polymers such as Poly (lactic acid) (PLA) and Poly(ε-caprolcatone) (PCL), which are widely used as biomaterials due to their complementing mechanical properties and degradation [7]. Along with having tailorable glass transition temperatures (Tg) and degradation rates by means of blending, they are also non-cytotoxic and possess shape memory, modifiable permeability and favor incorporation of growth factors [8,9,10]. Due to these virtues, our study attempts to use PLA/PCL blends as an alternative patch for MMC repair, possessing shape memory in addition to biodegradability and biocompatibility. Self-expansion at activation temperature (37ºC) would reduce time and difficulty level of sensitive surgery. Blend films of PLA and PCL were synthesized to attain a Tg of 37ºC for *in-vivo* expansion, and surface morphology was probed to account for the effect of surface properties on cell adhesion [11]. Liquid water permeability was measured using the water cup test method [12]. *In-vitro* cytotoxicity was studied using flow cytometry, to ensure complete absence of any toxic products in blend films; and cell viability was examined via fluorescence microscopy.

Methods: PLA pellets (4032D, NatureWorks LLC) and PCL microspheres (Capa™ 6506, Perstorp UK Ltd.) were blended by mixing overnight in Chloroform solvent (ACS LabChem), followed by drying cycles to form films. Surface morphology and thermal properties were measured using a FEI XL30 Scanning Electron Microscope and QA2000 Differential Scanning Calorimetry (10ºC/min from -40ºC to 200ºC) respectively. Shape memory tests were carried out in two humidity extremes: a) convection oven at 37ºC, b) water bath at 37ºC. For permeability tests, patch was sealed onto cup filled with water, and assembly weight was tracked for 25 hrs in 37ºC oven. The slope of weight loss vs. time was used to determine liquid water permeability. Human forearm fibroblast (HFF12) were cultured in 12 well plate till 80% confluency, followed by trypsinization and well plating (10^5 cells) at 37ºC in 2 exp. groups: 1) Control with no (-) blend film and 2) exposed to (+) blend film sections. After 5 days cells were tripsinized and labeled with PI and AnexinV flow cytometry analysis (LSRIII, BD Bioscience). *In-vitro* live/dead staining assay for cell viability was carried out using ReadyProbes® imaging kit (C/N R37609) and studied using fluorescence microscopy in the exp. group (+) blend film.

Results: The ideal formulation chosen based on thermal and surface properties, had a Tg (n=2) of 37.65±1.17ºC, and excellent surface finish without any signs of phase separation, indicating a partially miscible blend. Time of uncoiling via shape memory tests was ~55 seconds in scenario 'a', and ~3 seconds in scenario 'b'; though *in-vivo* tests would provide a better understanding of shape memory behavior. Liquid water permeability of patch was 0.000414µL cm^{-2} min^{-1} $H2O^{-1}$, comparable to negative controls in published studies, confirming a watertight barrier [12]. Flow cytometry data showed matching percentages of live cells for the control (-) (95.70±1.09%, n=4) and (+) blend film (95.24±0.94%, n=8) wells. Fluorescence microscopy images (Fig. 1) display fibroblasts growing on the film sections, indicating biocompatible surface properties.

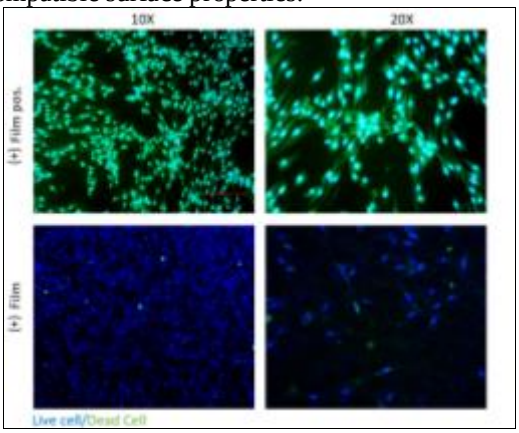

Figure 1. Fluorescence Microscopy images

Conclusions: Beneficial surface characteristics, favorable self-expansion times, confirmations of biocompatibility by cell culture; consolidate the validity of blend films as potential patch alternatives for MMC repair. Watertight barrier properties can potentially help in protecting the spinal cord, which might have a positive impact on neural regeneration. As future work, long-term patch performance will be studied in animal models.

References: 1) Mitchell, L. Lanc 2004; 364(9448):1885–1895; 2) Peiro, J.L. Surg Endosc (2013) 27: 3835; 3) Li, Z. Exp & Ther Med 2013; 5.5: 1531–1537; 4) Middleton, J. C. Biomat 2000; 21(23):2335-2346; 5) Hakimi, O. Int J Exp Path 2013; 94.4: 287–292; 6) Ikada Y. J. R. Soc 2006; (3):589-601; 7) Gunatillake, P. Biotech Ann Rev 2006; 12:301-347; 8) Tomihata, K. Poly Deg & Stab 1998; 59(1): 13-18; 9) Kronenthal, R L. Poly Sci & Tech 1975; (8):119–137; 10) Patrício, T. M. Proced CIRP 2013; 5:110-114; 11) Hallab, N.J. Tiss Engg. 2001, 7(1): 55–71; 12) Sword, R. J. Am J Dent. 2011; 24(1):20-4; 13)