Carboxyl Functionalized Polypyrrole for Surface Modification in Tissue Engineering

Jonathan Nickels, Francisco Serna, Joo-woon Lee, Christine Schmidt
The University of Texas at Austin

Statement of Purpose: Polypyrrole (PPy) has become a biomaterial of particular interest in recent years due to its electroactivity and biocompatibility. This work is furthering the goal of making polypyrrole a more useful material in tissue engineering applications. In particular, the potential of PPy in synthetic nerve grafts is a goal of this group. One of the prime limitations of PPy is the difficulty in modifying the surface. Overcoming this issue was approached by covalent modification of the pyrrole monomer to "tether" biologically active agents to the surface. The *N*-position is the target chosen for chemical modification. The concept of modification at the *N*-position is hardly a new one[1], however the tactic has found new interest in recent years[2]. The main contribution of this project will be the application of these ideas to tissue engineering problems.

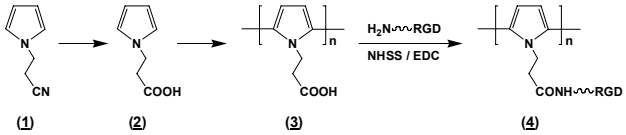

Figure 1. Reaction scheme in material preparation.

Methods: Synthesis of *N*-carboxy-ethyl-pyrrole (PyCOOH) began with commercially purchased N-cyano-ethyl-pyrrole being oxidized in the presence of KOH to the carboxyl form.[2] Confirmed was achieved by NMR, FT-IR, and XPS of the resulting polymer films. Two forms of the polymer were generated: a chemically synthesized powder, which can be pressed into pellets, and an electrochemically generated film. Conductivities were determined from the films and pressed pellets via the four point probe method. The *in situ* viability of the COOH functional group was shown via fluorescence microscopy. EDC/NHS chemistry was performed to attach a small amine-containing fluorescent marker to the film or powder surfaces. These surfaces were the viewed under a fluorescence microscope with the appropriate excitation wavelength. The same procedure was conducted to attach a short RGD containing sequence to the surface for cell studies.

Cell experiments were conducted using human umbilical vascular endothelial cells (HUVECs). These were seeded at 30,000 cells/cm^2 and cultured in both serum and non-serum conditions. The substrates for this experiment were standard PPy, unmodified Poly(N-carboxy-ethyl pyrrole) and an RGD modified version. Expanded cell studies are planned; specifically electro-stimulation studies and use of PC-12 cells.

Results / Discussion: Monomer synthesis was conducted successfully with an approximately 60 to 70% yield by mass. Data will be shown from the XPS and NMR which will show the successful synthesis of the monomer. XPS shows a characteristic up field shift of the C1s peak indicating the presence of the –COOH functional group.

The polymers synthesized via chemical and electrochemical methods were reacted with an amine containing fluorescent marker which was linked the -COOH functional group using EDC/NHS chemistry. Fluorescence microscope images show the presence of active functional groups (See Figures 2 A-C). The conductivity of the P(PyCOOH) was found to be four orders of magnitude lower than standard PPy, which is in agreement with published values[1].

The cell attachment studies show increased cell binding as well as cell spreading at the surface. Sufficient repetitions are still under way so that more quantitative claims may be made with statistical certainty. Quantitative assays (MTS) are also under way to quantify cell survival and viability at the surface.

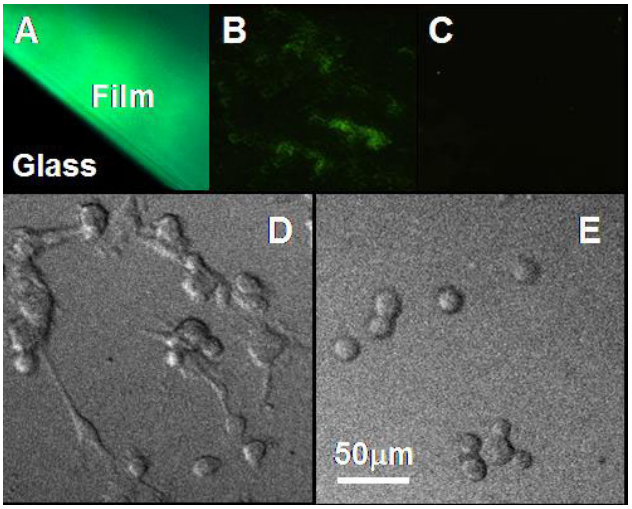

Figure 2. A and B show fluorescent microscopy images of the labeled modified P(PyCOOH), C shows a PPY control, D shows and phase contrast image of HUVECs on the RGD modified P(PyCOOH), E is a PPy control.

Conclusions: This study represents an investigation into the use of a carboxyl functionalized, N-substituted pyrrole for use in cell and tissue engineering applications. Synthesis of the monomer as well as synthesis and functionalization of the polymer were performed. These results, in conjunction with the increased cell adhesion to the RGD modified surface have generated enthusiasm to further pursue this material for tissue engineering applications. Future plans for this project include co-polymerizing the N-substituted monomer with regular pyrrole in varying molar ratios to improve conductivity. Conductivity is a critical factor in making this a uniquely useful biomaterial.

References:

1) Diaz, A.F. et. Al. *J. Electroanal. Chem. 1981,* **129** 115–132.
2) Azioune et Al. *Langmuir* 2004, 20, 3350.
3) Maeda, S et Al. , S. P. *Macromolecules*. 1995, 28, 2905-2911.