Summary

"Biocompatible, Biodegradable, and Electroactive Polyurethane-Urea Elastomers with Tunable Hydrophilicity for Skeletal Muscle Tissue Engineering"

Leading Authors: Baolin Guo, Peter X. Ma

1. Objective:

-The objective is to focus attention on the research of degradable Polyurethanes (PUs) with good **electroactivity for tissue repair**. They have excellent soft tissue-like properties which can be promising that **electroactive PUs** can be used for regeneration materials **for soft tissue such as muscles and nerves which respond to the electrical signals**.

-From previous studies, it indicated that **cell proliferation and differentiation can benefit from an electrical stimulus**; therefore, **conductive or electroactive polymers can conduct electricity to influence cell behavior** in biological environments, specifically for cell cultures used in research and medical applications.

> Cell Proliferation: The rate at which cells multiply can be increased or controlled.
>
> Cell Differentiation: Cells can be influenced to mature into a specific type of cell, which is particularly useful in tissue engineering where specific cell types need to be grown.

2. Background:

-The main challenge addressed in this article is development of electroactive and elastic biomaterials that **mimic soft tissue elasticity and influence cell behavior during tissue regeneration**. Traditional polyurethanes (PUs) have diverse compositions and flexibility, making them suitable for various biomedical applications. However, the need for electroactive properties has directed research towards **combining polyurethanes with conductive polymers.**

3. Process:

3.1 Materials to synthesize the electrostatic PUU

-The synthesized electroactive PUU copolymers were formulated from **amine-capped aniline trimer (ACAT)**, **dimethylol propionic acid (DMPA), polylactide**, and **hexamethylene diisocyanate (HDI)**. These materials were **chosen to provide the desired properties of elasticity, hydrophilicity, and degradability**, with a **focus on enhancing surface properties for better cell interaction**.

3.2 Methods and Synthesis

- The synthesis involved a two-step polymerization process:

(1) Mixing PLLA and DMPA and subjecting them to dehydration and dissolution steps.

> This step forms the base of the polymer matrix. PLLA (polylactide) provides the biodegradable backbone, while DMPA (dimethylol propionic acid) enhances the hydrophilicity of the copolymers. This phase sets the stage for the second reaction by ensuring the reactants are in the correct form and concentration, improving the reaction's efficiency and the properties of the final product.

(2) Adding ACAT and HDI under nitrogen to form the prepolymers, followed by precipitation to obtain the PUU copolymers.

> ACAT (amine-capped aniline trimer) is introduced at this stage as an electroactive compound to impart desired electrical properties to the polymer, which are critical for applications like tissue engineering where electrical conductivity can influence cell behavior.
>
> HDI (hexamethylene diisocyanate) acts as a linker that reacts with the components to form prepolymers. Performing this step under nitrogen prevents unwanted reactions with atmospheric moisture or oxygen, which could interfere with the intended chemical reactions.
>
> The prepolymers are then precipitated, which means they are transformed from a dissolved state into a solid form. This step is crucial for isolating the PUU copolymers from the reaction mixture, allowing them to be collected and purified.

-The **electroactivity of the PUU copolymers** were studied by **UV-vis spectroscopy** and cyclic voltammetry

-Hydrophilicity of the copolymer films was tuned by changing DMPA content and doping of the copolymer. Cytotoxicity of the PUU copolymers was evaluated by mouse C2C12 myoblast cells.

> **DMPA** •• Dimethylol Propionic Acid •• DMPA is introduced into the polyurethane-urea (PUU) copolymers to improve their surface hydrophilicity, which is crucial for enhancing cell adhesion and interaction. This modification is part of the copolymer's design to make them more suitable for biomedical applications, particularly in tissue engineering where surface properties significantly influence cell behavior.
>
> **Cytotoxicity** tests are conducted to ensure that the materials do not negatively affect cell viability or function. This involves exposing cells to the material and then measuring various indicators of cell health, including cell membrane integrity, enzymatic activity, and other metabolic functions.

4. Results / Equations:

4.1. Evaluation of copolymer degradation under specific conditions over a set period:

$$Weight\ Loss\ (\%) = \frac{W_0 - W_t}{W_0}$$

$W_0 = Original\ weight\ of\ copolymer\ film$

$W_t = Dry\ weight\ of\ specimen\ after\ degredation$

> **The goal in tissue engineering is for the degradation rate of the scaffold material to ideally match the rate of tissue regeneration*

4.2. Synthesis of Copolymers

“ACAT is introduced into the copolymers to provide the electroactivity of the materials because of its native electrochemical behavior. DMPA with a pendent carboxyl group is employed to improve the hydrophilicity of the materials. All the (macro)monomers are connected together with HDI by

polyaddition. Because of the high reaction activity of isocyanate with hydroxyl and amine groups, the PUU copolymers are obtained with a high yield. With an equal feed molar ratio of ACAT and DMPA, the weight fractions of them in the copolymers increase with the decrease of the PLLA molecular weight. Furthermore, six copolymers (PUUH0-5) based on PLLAH are synthesized to study the effect of DMPA content on the hydrophilicity of copolymer avoiding the influence from the length of the PLLA chains."

••

The success of the synthesis and structure of the PUU copolymers and demonstrated by **FT-NMR (Fourier Transform)** and **H-NMR (Hydrogen) analysis**.

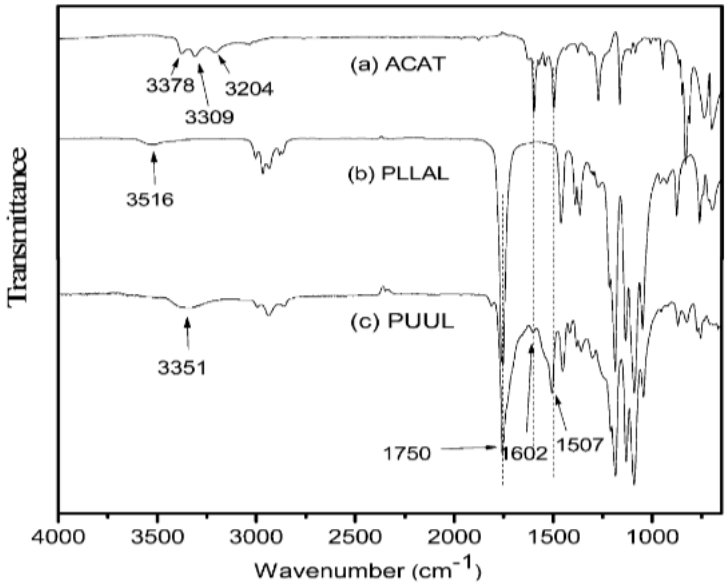


Figure 1. FT-IR spectra of (a) ACAT, (b) PLLAL, and (c) PUUL.

Figure 1. is an FT-NMR:

CURVE (A): Spectra of ACAT

This shows characteristic peaks associated with its specific chemical structure, likely reflecting the presence of amine groups and aromatic rings, which are typical in conductive polymers.

CURVE (B): Spectra of PLLAL:

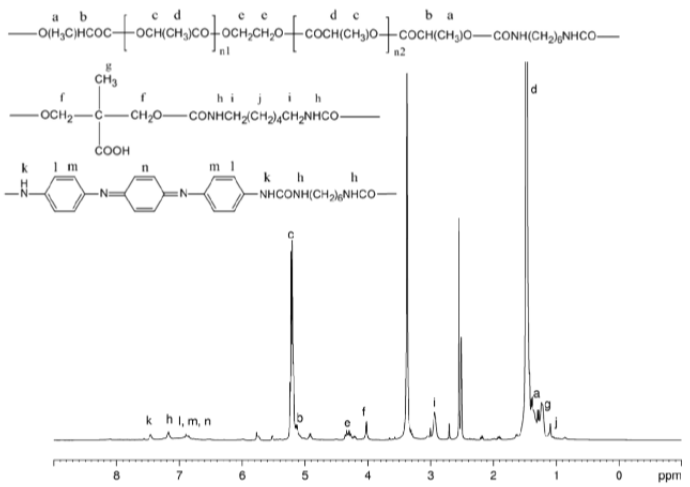
Indicates the presence of carbonyl groups typical of polyester (from the lactide), and other peaks that would be associated with the backbone of the polymer.

Lactide: composed of two lactic acid molecules with the removal of water. It is the precursor used in the production of the biodegradable polymer, polylactic acid (PLA).

CURVE (C): Spectra of PUUL:

Shows a combination of features from both ACAT and PLLAL, along with additional peaks that suggest the formation of urea linkages (possibly from the reaction between amine groups in ACAT and isocyanate groups in HDI used in the synthesis). The spectrum also indicates the formation of hydrogen bonds between the ester, amide, and urea groups in the copolymer, which are essential for providing the elasticity and mechanical integrity required for biomedical applications.

Figure 2. is an H-NMR of the PUUL Polymer:



Analysis:

(1) A sharp peak at 7.45 ppm (singlet, 2H), which corresponds to -NH- groups.

(2) Two doublet peaks at 6.90 ppm and 6.84 ppm (each 2H), representing aromatic hydrogen (Ar-H).

(3) A sharp peak at 6.80 ppm (singlet, 2H) also corresponding to aromatic hydrogen.

Properties:

(1) Formation and Stability of the Copolymer:

The -NH group is crucial in the formation of urethane and urea linkages during the polymerization process which introduce sites for hydrogen bonding. These linkages result from the reaction between isocyanate groups (NCO) and hydroxyl or amine groups, contributing to the chemical stability and integrity of the polymer structure.

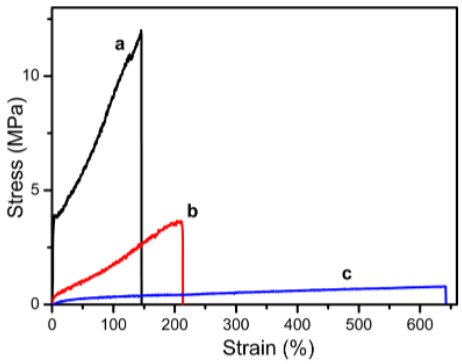
(2) Physical Cross Linking and Elasticity:

The presence of -NH groups allows for the formation of hydrogen bonds between different polymer chains. These hydrogen bonds act as physical cross-linking points which enhance the elasticity of the material, making it suitable for applications that require flexible yet durable materials, such as muscle tissue engineering scaffolds.

(3) Interactions between biological systems:

The aromatic hydrogen (Ar-H) groups in the ACAT segment of the copolymers affect the polymer's interaction with biological systems. These groups can influence the material's biocompatibility and its interaction with cellular components, which is critical for its application in biomedical engineering.

Figure 4. Stress-Strain Curves of (a) PUUH, (b)PUUM, and (c)PUUL copolymer



"The high breaking elongation rate of these PUU copolymers is attributed to the hydrogen bonds between ester, urethane, and urea groups which serve as physical cross-linking points to enhance the intra- and intermolecular interactions between the PUU macromolecules."

Figure 5. Degradation profile of copolymer films at 37°

Analysis:

-The rate of in *vitro* degradation is significantly influenced by the length of PLLA segment and the concentration of urethane/urea groups ***[Discussed in figure 2]***

-PUUL degraded continuously from the start and lost 100% of their weight is 25 days

-PUUM shows a slow degradation speed at first 15 days with a weight loss of < 15%

-Remaining mass of PUUH was still > 90& after a month.

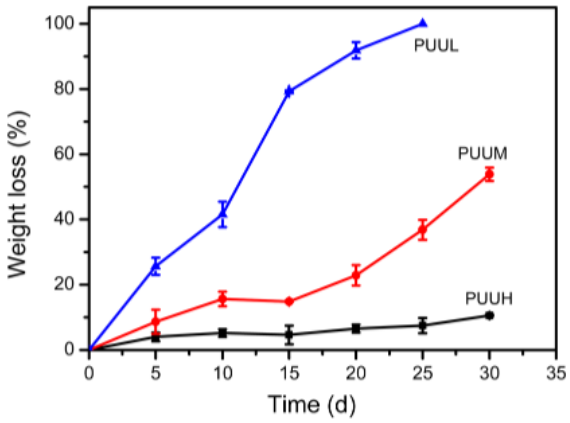


Figure 6. Cell Viability of C2C12 cells on ***TCP, *PLLA, *PU (*Control Groups*)**, PUUH, PUUM, and PUUL copolymer substrates

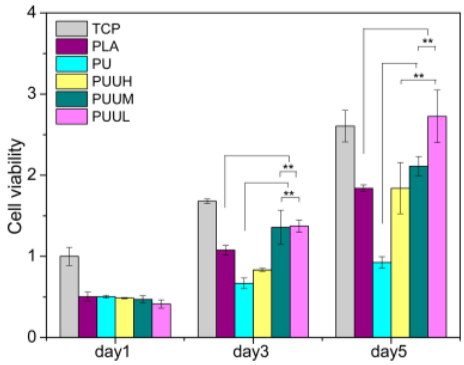


Figure 6. Cell viability of C2C12 cells on TCP, PLLA, PU (Tecoflex), PUUH, PUUM, and PUUL copolymer substrates. TCP, PLLA, and PU (Tecoflex) groups served as the control groups. Note: (**) for $p < 0.01$.

Figure 8, Statistic data of myotubes after 7 days of culture for myogenic differentiation of C2C12 cells: (a) myotube number per 10 5 µm2 , (b) myotube length, (c) myotube diameter, and (d) myotube maturation index

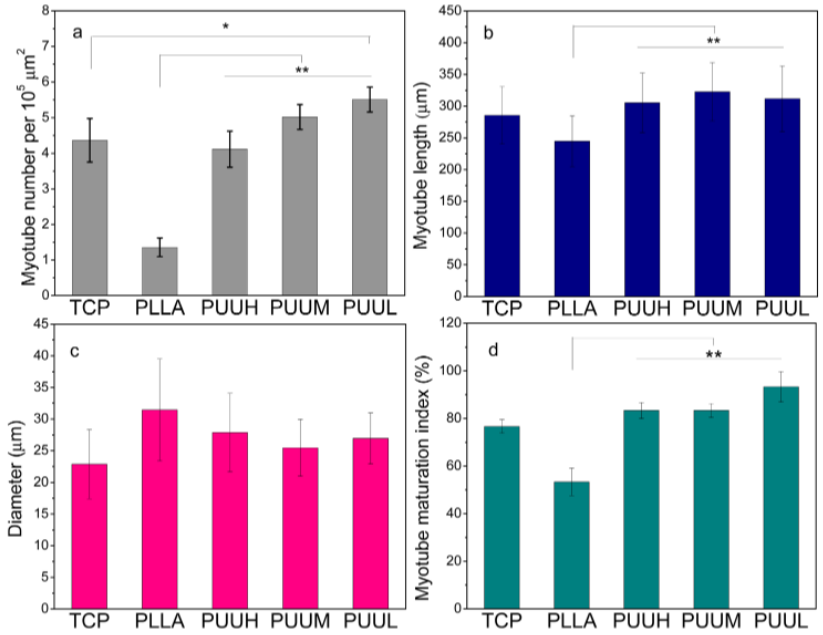


Figure 8. Statistic data of myotubes after 7 days of culture for myogenic differentiation of C2C12 cells: (a) myotube number per 10^5 μm^2, (b) myotube length, (c) myotube diameter, and (d) myotube maturation index (% myotubes with ≥ 5 nuclei). Note: (*) for $p < 0.05$; (**) for $p < 0.01$.

Myotubes:

-Specialized cell that forms during the development of muscle tissue.

-Created from the fusion of multiple myoblasts, which are the precursor cells that give rise to muscle cells.

Role in Regeneration:

-Following muscle injury, the regenerative process involves the activation of satellite cells (a type of myoblast), which proliferate and eventually fuse to form new myotubes that help restore the damaged muscle.

Analysis:

(1) Myotube Number per $105\mu m^2$

Significant increase in the number of myotubes for samples treated with PUU copolymers compared to PLLA. This increase is a direct measure of the effectiveness of PUU materials in promoting myogenic differentiation, indicating the optimal conditions provided by its formulation for muscle cell growth and development.

(2) Myotube Length

This metric gives the average length of the myotubes formed, where PUU copolymers promote the formation of longer myotubes compared to controls, enhancing the structural integrity and potential functionality of the muscle tissue being modeled.

(3) Myotube Diameter

It compares the thickness of myotubes across different samples, helping to understand the robustness of the muscle fibers developed.

(4) Myotube Maturation Index

This percentage indicates the maturity of myotubes, with those containing more than 5 nuclei considered more mature. The data reveals that the maturation index is higher in PUU samples compared to PLLA, with PUUL again showing superior performance.

Formation of Myotubes:

(1) Myoblast Differentiation

In the early stages of muscle development, myoblasts (muscle stem cells) proliferate and align with each other.

(2) Cell Fusion**

These myoblasts then fuse together to form a multi-nucleated cell known as a myotube. This process is critical for the maturation of muscle fibers.

(3) Maturation into Muscle Fibers

Myotubes further mature and eventually develop into fully functional muscle fibers, which are the contractile units of muscle tissue.

Figure 9. Myogenin (MyoG) and troponin T1 (TNNT) gene expression of C212 cells on different substrates at day 7

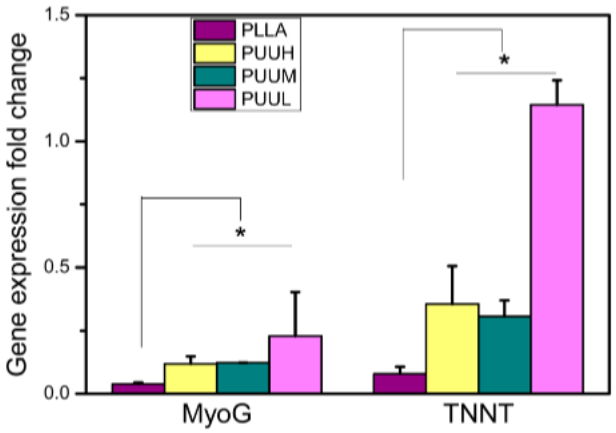


Figure 9. Myogenin (MyoG) and troponin T1 (TNNT) gene expression of C2C12 cells on different substrates at day 7. Note: (∗) for $p < 0.01$.

References

Jignesh P. Sheth, Serkan Unal, Emel Yilgor, Iskender Yilgor, Frederick L. Beyer, Timothy E. Long, Garth L. Wilkes, A comparative study of the structure–property behavior of highly branched segmented poly(urethane urea) copolymers and their linear analogs, Polymer, Volume 46, Issue 23, 2005, Pages 10180-10190, ISSN 0032-3861, https://doi.org/10.1016/j.polymer.2005.07.068. (https://www.sciencedirect.com/science/article/pii/S0032386105011067)

Brunette, C. M., Hsu, S. L., & MacKnight, W. J. (1982). Hydrogen-bonding properties of hard-segment model compounds in polyurethane block copolymers. Macromolecules, 15(1), 71–77. https://doi.org/10.1021/ma00229a014 (https://pubs.acs.org/doi/pdf/10.1021/ma00229a014)

Ruixuan Lv, Shuo Jin, Lei Li, Qian Wang, Lele Wang, Jin Wang, Jingshuai Yang, The influence of comonomer structure on properties of poly(aromatic pyridine) copolymer membranes for HT-PEMFCs, Journal of Membrane Science, Volume 701, 2024, 122703, ISSN 0376-7388, https://doi.org/10.1016/j.memsci.2024.122703. (https://www.sciencedirect.com/science/article/pii/S0376738824002977)

Jignesh P. Sheth, Serkan Unal, Emel Yilgor, Iskender Yilgor, Frederick L. Beyer, Timothy E. Long, Garth L. Wilkes, A comparative study of the structure–property behavior of highly branched segmented poly(urethane urea) copolymers and their linear analogs, Polymer,

Volume 46, Issue 23,
2005,
Pages 10180-10190,
ISSN 0032-3861,
https://doi.org/10.1016/j.polymer.2005.07.068.
(https://www.sciencedirect.com/science/article/pii/S0032386105011067)

Mainak Das, John W. Rumsey, Neelima Bhargava, Maria Stancescu, James J. Hickman,
Skeletal muscle tissue engineering: A maturation model promoting long-term survival of myotubes, structural development of the excitation–contraction coupling apparatus and neonatal myosin heavy chain expression,
Biomaterials,
Volume 30, Issue 29,
2009,
Pages 5392-5402,
ISSN 0142-9612,
https://doi.org/10.1016/j.biomaterials.2009.05.081.
(https://www.sciencedirect.com/science/article/pii/S0142961209005948)