Tyrosine-derived polycarbonate scaffolds for bone regeneration in the craniofacial complex

Maria Hanshella R. Magno[1], Jinku Kim[2], Abiraman Srinivasan[2], Aniq Darr[1], Jeffrey Hollinger[2] and Joachim Kohn[1]

[1] New Jersey Center for Biomaterials, Rutgers – The State University of New Jersey, Piscataway, NJ USA

[2] Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, PA USA

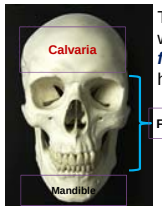
Introduction

In a review of around 28,000 patients injured in Afghanistan and Iraq over a period of almost three years, from September 11, 2001 to January 1, 2004, head and neck injuries ranked second to extremity injuries in terms of number of incidence[1]. Although the head and neck only comprise 12% of the body surface area, 64% of combat deaths are due to these injuries[2] .

There is a need to salvage and regenerate damaged and lost tissues of those that survive from these injuries so as to avoid lifelong complications and so that these soldiers can go back to a life that is productive[2]. Current, treatment options include autografts, metallic implants, ceramics and non-biodegradable synthetic polymers. The use of biomaterials for tissue engineering is an emerging technology that offers hope and promise of regeneration of diseased or lost tissues. One of the desirable characteristics of these biomaterials for regeneration of tissues is their tunability of physicochemical properties by modifying their structure, composition and architecture. One such class of biomaterial is the **tyrosine-derived polycarbonates**, which have shown potential as orthopedic implant material as observed in different animal models:

- in the distal femur and proximal tibia of rabbits[3,4]
- in a canine bone chamber model[5]
- in the calvaria of rabbit[6]
- in rabbit mandibular defects[7,8]

Objective

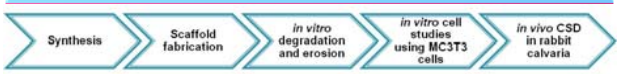


http://www.gwc.maricopa.edu/cass/bio201/skull/

To ***design***, ***develop*** and ***provide therapies*** for the wounded war-fighter that will ***regenerate form*** and ***function*** to ***calvarial*** anatomy by fabricating scaffolds that have the following properties:

- osteoconductive
- as strong as bone
- degradable and resorbable
- can deliver growth factors, drugs and/or cells

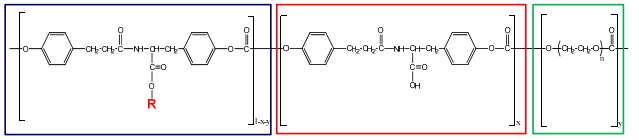
Method



A schematic representation of the research method is shown above. Briefly, tyrosine-derived polycarbonates (**Tyr-PC**) are synthesized through a polycondensation reaction with triphosgene/pyridine in dichloromethane. Scaffolds are fabricated using a combination of solvent casting/particulate leaching technique. Properties of scaffolds with or without growth factors and/or cells are determined by *in vitro* cell studies using MC3T3-E1.4 cell line and in *in vivo* rabbit calvarial defect model.

Results

Polymer synthesis



poly(DTR-co-x%DT-co-y%PEG$_{MW}$ carbonate)

Figure 1: *Tyrosine-derived polycarbonate terpolymer platform technology.*

Table 1: *A summary of effects of the components of tyrosine-derived polycarbonates on polymer properties.*

DTR	DT	PEG
hydrophobic; provides strength; slow degradability; mediates protein adsorption	more hydrophilic; accelerates resorption; negative surface charge	affects cell attachment, migration & proliferation; protein adsorption

Variations in the composition of the Tyr-PC terpolymer (**Fig. 1**) result in changes in the properties of the resulting polymer. **Table 1** shows how each component of the terpolymer can modify its properties.

Scaffold fabrication

SEM image of the scaffold (**Fig. 2**) shows:

- a bimodal pore distribution of macropores (200 – 400 μm) and micropores (<100 μm)
- a highly oriented alignment of micropores around the macropores
- an interconnected and very open architecture of the scaffold.

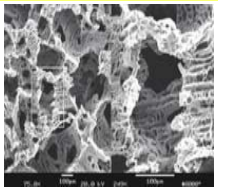


Figure 2: *SEM image of a scaffold at 75x and 250x magnification.*

in vitro degradation and erosion in PBS at 37 ^{0}C

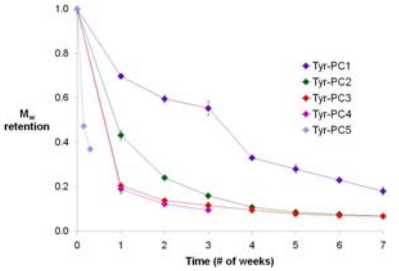


Figure 3: *M_w retention of Tyr-PC scaffolds at different timepoints.*

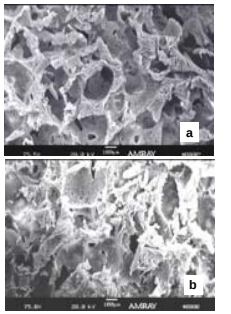


Figure 5: *SEM images of scaffolds at t=0 (**a**) and t=4 (**b**) weeks.*

Error bar represents mean ± standard deviation for n=3

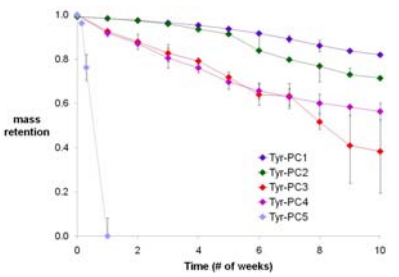


Figure 4: *Mass retention of Tyr-PC scaffolds at different time points.*

- Changing the composition of the terpolymer resulted to changes in the rate of degradation and erosion.
- *In vitro* degradation and erosion of various Tyr-PC scaffolds in PBS at 37^{0}C showed considerable polymer backbone degradation (**Fig. 3**) but minimal mass loss (**Fig. 4**).
- The open and interconnected pore architecture of Tyr-PC scaffold collapsed due to degradation (**Fig. 5**).

in vitro cell studies

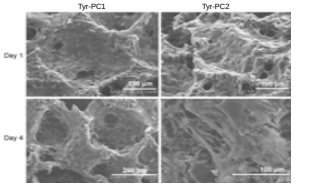


Figure 6: *SEM images of MC3T3-E1 cells seeded on Tyr-PC scaffolds after day 1 and day 4 of culture.*

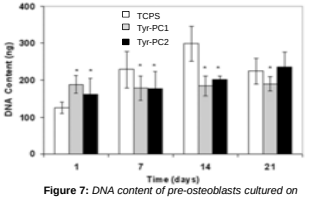


Figure 7: *DNA content of pre-osteoblasts cultured on different substrates at various time periods.*

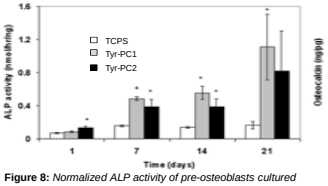


Figure 8: *Normalized ALP activity of pre-osteoblasts cultured on different substrates at various time periods.*

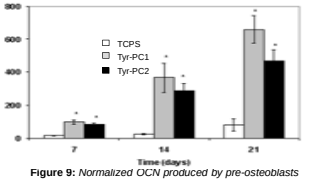


Figure 9: *Normalized OCN produced by pre-osteoblasts cultured on different substrates at various time periods.*

*Error bar represents mean ± standard deviation for n=3 and * represents significant difference (p<0.05) compared to the control (TCPS).*

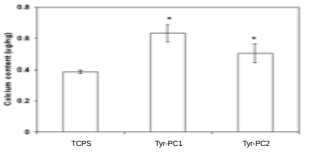


Figure 10: *Mineralized calcium deposition of the ECM at day 21 produced by pre-osteoblasts cultured on different substrates.*

Overall Tyr-PC scaffolds:

- showed robust MC3T3-E1.4 cell attachment (**Fig. 6**)
- promoted osteoblast differentiation with significantly greater amounts of ALP, OCN and calcium deposition on the Tyr-PC surface compared to 2D tissue culture plates (TCPS) (**Fig. 7-10**)

in vivo defect in the rabbit calvaria

Figure 11: *Empty defect at 8 weeks (**a**) vs. a defect with Tyr-PC co-polymer scaffold at 16 weeks (**b**). (Stains: Stevenel's Blue and Van Gieson's Picrofuchsin)[6]*

In a delayed healing defect (8 mm) model in rabbit calvaria, Tyr-PC scaffold was able to bridge the defect totally in 16 weeks (**Fig. 11b**) and the healing caused by Tyr-PC scaffold was more pronounced than in empty defects (**Fig. 11a**).

Figure 12: *Creating a CSD in an adult New Zealand White rabbit.*

Using a trephine, a 15 mm defect was drilled on the skull of the rabbit. A Tyr-PC scaffold was implanted (15 mm (diameter) x 2.5 mm (thickness)) (**Fig. 12**). *In vivo* biocompatibility and osteoconductive properties of the scaffold will be evaluated after 8 and 12 weeks of implantation by micro-CT and histology. This experiment is on-going.

Conclusion

- Varying the composition of the terpolymer has a clear effect on the rate of *in vitro* degradation and erosion.
- *In vitro* cell studies on MC3T3 cells show that highly porous scaffolds fabricated from tyrosine-derived polycarbonate are biocompatible and enhance production of ALP, OCN and calcium by pre-osteoblasts.
- *In vivo* delayed healing defect in the rabbit calvaria shows that Tyr-PC scaffold repaired the gap more effectively than an pty defect.

References

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The Rutgers Cleveland Clinic Consortium of AFIRM

RCCC_AFIRM@biology.rutgers.edu

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