

## In vitro integration of human skin dermis with porous cationic hydrogels

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### Abstract

Porous poly(DMAA-co-AMTAC) hydrogels, fabricated using the inverted colloid crystal method, were used to observe their integration with human skin. Full thickness human breast skin explants discarded from surgeries were cultured for up to 10 days at the air–liquid interface using a Transwell culture system. Cylindrical, disk- or other shaped hydrogels were placed inside the skin explants fitting punctures produced by punch biopsies or scalpels and full section histological analysis of the skin explants with the inserted hydrogel was then performed. In addition, separated hydrogels were cultured up to 7 days with human fibroblasts. The results indicate that poly(DMAA-co-AMTAC) hydrogels induce substantial extracellular matrix material deposition, maintain dermal integrity in the contact areas with the skin and permit dermal fibers to integrate into the hydrogel pores. Different types of cells remaining in the explants migrated into the hydrogels pores, including red blood cells. Fibroblasts adhered to and colonized separately cultured hydrogels. We plan to use this type of soft material as an interface to permit skin integration with percutaneous devices in contact with skin.

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### 1. Introduction

There has been a steady increase in the number of medical procedures with permanently or temporarily implanted percutaneous devices. While totally implanted devices have been successfully used for several years [1], percutaneous devices, defined as those that permanently cross the skin to perform their function, have been less successfully developed. Following in part from the ideas of Branemark [2], recent research has led to the development of a perma-

nently implanted prosthetic, attached directly to the bone. While some of the challenges have been addressed, one substantial problem limiting the development of such devices is the presence of chronic irritation, inflammation and infection [3–5], which is associated with implanting a device through the skin and is derived, in part, from the imperfect seal between an organic tissue and the inorganic frame of the device [6]. Infection rates depend on the type of device [7], but the costs, in economic and human terms, are very high [8,9]. While skin inflammation and infection constitutes a major hurdle in the development of permanent biointegrated prosthetic devices, the same problems exist with smaller or temporary devices that are either already in clinical use, like catheters and fixator pins [4], or are being actively developed, like glucose sensors [10].

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These devices show high variability in their intrinsic properties, with a disparate number of material composition, surface structure, porosities and topologies [11]. This variability hampers the investigation of a general solution for this unresolved problem.

Normally, percutaneous devices are made of hard materials where the soft tissue need to attach and integrate, thus there is a transition between two elements of very different chemical and mechanical properties. We propose that the use of soft materials, for instance in the form of hydrogels, forming a preliminary area in contact with skin would allow better initial skin integration. For this reason, we sought to use a soft, biointegrative material allowing dermal integration that could be produced as hydrogels. We have been investigating the use of *N,N*-dimethylacrylamide (DMAA) copolymerized with (3-acrylamidopropyl)-trimethylammonium chloride (AMTAC) (poly(DMAA-co-AMTAC)) for its possible use as an interface in the ulterior biointegration of human skin with percutaneous devices. This type of hydrogel has been employed in gene therapies as vectors for DNA and oligonucleotide delivery [12], and was shown to promote cell adhesion [13,14], partially due to its cationic nature.

Given that cellular response is different in two- and three-dimensional scaffoldings [15], the poly(DMAA-co-AMTAC) hydrogels were constructed as three-dimensional tissue culture scaffolds. Although three-dimensional tissue culture matrices have been used, for instance by rapid prototyping [16], they have certain limitations, particularly in their physical dimensions. The introduction of three-dimensional (3-D) hydrogel scaffoldings of inverted colloidal crystal (ICC) topology made from poly(acrylamide) hydrogels [17] overcame these limitations, providing an easy control of their porous geometry. In contrast to poly(acrylamide) scaffoldings, the poly(DMAA-co-AMTAC) hydrogels we have used have the advantage that they do not require surface modification to allow better cell adhesion.

To evaluate this soft material as an inductor of whole skin integration, the poly(DMAA-co-AMTAC) hydrogels were tested in different ways. First, whole human skin was cultured with the hydrogels and the reaction when integrated to the hydrogels was studied. In this case, the hydrogels were cultured using human organotypic breast skin explants discarded from surgeries at the air–liquid interface using a Transwell culture system. Secondly, isolated human fibroblasts were cultured inside the hydrogels and their viability was assessed. Finally, the poly(DMAA-co-AMTAC) polymer was deposited as thin films on coverslips and the adhesion of human fibroblasts was analyzed. These results are presented in this paper.

## 2. Materials and methods

### 2.1. Skin preparation

Full thickness human breast skin explants from discarded material from surgeries performed at the University of Michigan Health System were used. The specimens were

received from healthy human subjects after informed consent and immediately prepared for culture. After removal of subcutaneous fat, the tissue was rinsed abundantly with  $1 \times$  PBS containing  $125 \mu\text{g ml}^{-1}$  of gentamicin (Invitrogen/GIBCO, Carlsbad, CA) and  $1.87 \mu\text{g ml}^{-1}$  of amphotericin B (Sigma–Aldrich, Milwaukee, WI) and placed in aliquots of the same medium in an incubator at  $37^\circ\text{C}$  for 2 h, with change of medium after 1 h. The culture medium used was EpiLife (Cascade Biologics, Portland, OR), supplemented with EpiLife defined Growth Supplement EDGS (Cascade Biologics, Portland, OR). In addition, the medium was supplemented with  $75 \mu\text{g ml}^{-1}$  of gentamicin and  $1.125 \mu\text{g ml}^{-1}$  of amphotericin B. The final concentration of calcium used in the culture medium was 1.2 mM. After preparation, skin specimens of approximately  $1.5 \text{ cm}^2$  were cut using a scalpel and cultured for 5 or 10 days at  $37^\circ\text{C}$  in a 5%  $\text{CO}_2$  atmosphere, epidermal side up at the air–liquid interface, in a Transwell system consisting of 6-well Transwell carriers (Organogenesis, Canton, MA) and six Corning Costar supports (Fisher Scientific, Pittsburgh, PA). Culture medium was changed every 24 h and the stratum corneum remained constantly exposed to the air.

### 2.2. Preparation of 3-D ICC poly(DMMA-co-AMTAC) hydrogel scaffoldings

The following materials were used for the hydrogel synthesis: the neutral monomer *N,N*-dimethylacrylamide ( $\text{CH}_2=\text{CHCON}(\text{CH}_3)_2$  (DMAA), Aldrich), the cationic monomer (3-acrylamidopropyl)-trimethylammonium chloride ( $\text{H}_2\text{C}=\text{CHCONH}(\text{CH}_2)_3\text{N}(\text{CH}_3)_3\text{Cl}$  (AMTAC), Aldrich), the cross-linker *N,N'*-methylenebisacrylamide ( $(\text{CH}_2=\text{CHCONH})_2\text{CH}_2$  (NMBA), Sigma) and the free radical initiator potassium persulfate ( $\text{K}_2\text{SO}_4$  (KPS), Sigma). Deionized distilled (DDI) water (E-pure, Barnstead) was used to make the pre-polymer solution and the free radical solution. An aqueous suspension of polystyrene (PS) microspheres (Duke Scientific,  $3 \times 10^4$  particles per ml with 1.4% size distribution) was used for the construction of the colloidal crystal.

The poly(DMAA-co-AMTAC) was prepared from co-polymerization between DMAA and AMTAC in an aqueous environment. The monomer chains were chemically cross-linked by NMBA, and the polymerization was triggered by the free radical initiator KPS. In detail, a series of DMAA and AMTAC ratios (variation in %A while keeping %T constant) dissolved in DDI water were mixed with a series of %C in a 20 ml glass vial. Either nitrogen or argon gas was continuously purged so as to maintain an oxygen-free environment. The mixture was stirred vigorously for 30 min and then partitioned into 4 ml glass vials (1 ml in each vial). Aqueous KPS solution (3 wt.%) was then added at a ratio of 1:10 by volume into the partitioned mixtures to set off the polymerization. The glass vials of precursors were kept in an oven at  $75^\circ\text{C}$  for 3 h and at  $60^\circ\text{C}$  overnight to aid the formation of the gels.

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