Description

Title

§X+1 Continuous IL-6 Sensor Module

X+1.1 Sensing Principle and Kinetics Justification

The IL-6 sensor employs an **electrochemical aptamer-based sensing** principle to achieve continuous, real-time detection of IL-6. A thiolated DNA aptamer specific to IL-6 (a ~21 kDa proinflammatory cytokine) is immobilized on the sensor's microelectrodes and labeled with a redox reporter (methylene blue). When IL-6 binds, the aptamer undergoes a conformational change that modulates electron transfer, producing a quantifiable voltammetric currentpubmed.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. This label-free binding-transduction approach (often termed an aptamer-FET or E-AB sensor) is **inherently reversible**, allowing IL-6 molecules to bind and unbind repeatedly so that the sensor can track concentration changes continuously. This is critical because high-affinity protein receptors (e.g. antibodies) often sequester targets for hours ($t_{½} \approx 20$ h), blunting responsiveness to decreasing levelspubmed.ncbi.nlm.nih.gov. By contrast, the chosen aptamer has a dissociation constant in the low-nanomolar range, balancing sensitivity with a manageable complexation half-life. As a further safeguard, the module uses a brief **active electrochemical "reset"** after each measurement cycle – a high-frequency potential pulse that accelerates ligand dissociation – regenerating the unbound aptamer within ~1 minutepubmed.ncbi.nlm.nih.gov. This ensures that when IL-6 levels fall (for example, after therapeutic intervention), the sensor can promptly recover and reflect the decrease, rather than remaining saturated. The sensor's kinetics are thus matched to IL-6's known rapid dynamics: IL-6 can surge and peak within ~2 hours during sepsismdpi.com, far earlier than conventional markers (CRP, PCT). The aptamer-FET design, with its real-time electron transfer readout, captures these swift IL-6 excursions with minimal latency (sub-minute electrochemical integration), providing the TraceLoop controller timely data to mitigate cytokine storms. Alternative transduction modes were evaluated – including label-free immuno-FETs and sandwich immunoassays – but the **aptamer-based electrochemical approach** was selected for its combination of continuous operation, single-step detection (no exogenous reagents), and attomole-level sensitivity in small volumespubmed.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. This strategy has been validated in recent prototypes that simultaneously measured IL-6 via aptamer sensors in microneedle arrayspubmed.ncbi.nlm.nih.gov, demonstrating robust performance in serum and ISF with negligible signal drift over hours of continuous usepubmed.ncbi.nlm.nih.gov.

X+1.2 Physical Form Factor and Wear Site

Physically, the IL-6 sensor module is implemented as a **dissolvable microneedle patch** (approx. 10 mm × 10 mm) that mounts to a low-profile connector on the patient's skin. The patch contains a **1 cm² array of**

micron-scale needles made of biocompatible polymer. Upon application to the upper arm (over the deltoid or lateral tricep skin), 100+ microneedles (~500 μm length, 300 μm base width) painlessly penetrate the stratum corneum. This creates an array of microchannels into the dermal interstitial fluid, without reaching blood capillaries or causing bleeding. Within **5–10 minutes of placement**, the needles **hydrate and dissolve** in the tissue fluid, leaving behind open microchannels that continuously interface ISF to the sensor's electrode array. The dissolvable needles serve two purposes: (1) they **painlessly breach the skin barrier** (needle tips are sharper than 30G, and insertion is below pain receptor thresholds), and (2) by dissolving, they eliminate any retained sharp or rigid materials, enhancing safety and comfort during the 24 h wear period. The patch is designed for **single-use 24 hour wear**: after a day of monitoring, it is removed and discarded with the microneedles substantially dissolved (any residual polymer is biocompatibly absorbed). The wear site (upper arm) was chosen for adequate perfusion and patient tolerance – it provides a consistent ISF composition representative of systemic IL-6mdpi.com, a relatively uniform skin thickness for reliable microneedle penetration, and is convenient for caregivers to access. The patch attaches via an **integrated medical adhesive backing** that seals the perimeter, preventing contamination and aiding consistent hydration of the sensor. Overall form-factor thickness is under 3 mm (excluding needles) to lie flat on the skin and avoid snags. The module connects to the TraceLoop controller via a **flexible tail** that seats in a zero-insertion-force (ZIF) socket on the main unit (or a telemetric skin hub). This substrate "tongue" and adhesive keep the patch secure during patient movement. The **hot-swap design** allows the patch to be applied and removed without powering down the system; the controller auto-detects the module's presence and validity upon connection. In summary, the IL-6 sensor's physical design – a daily-replaceable microneedle patch on the arm – provides a minimally invasive, robust interface to the patient's ISF compartment, enabling continuous cytokine monitoring with negligible discomfort and no permanent indwelling lines.

X+1.3 Layered Fabrication and Materials *(Figure 5)*

Figure 5 illustrates the layer-by-layer construction of the IL-6 microneedle sensor patch, which integrates multiple functional strata in a stacked assembly:

Figure 5 – Layered Microneedle Patch Stack for IL-6 Sensing. *The exploded schematic shows the functional layers of the continuous IL-6 sensor patch (not to scale), from the skin-contact needles up to the electrical connector. Each layer's composition and role are summarized below:*

Layer	Primary Materials & Geometry	Function	Design Remarks

1. Substrate & Connector *see **FIG. 5-1*** ***Layered microneedle-patch stack (exploded view)***	25 µm flexible polyimide film with embedded gold traces; one end terminates in a reinforced **docking tongue** (ZIF contact pads).	Provides the mechanical support and electrical routing for the sensor. The polyimide substrate spans the 1 cm^2 patch area, carrying signal traces from each sensor electrode to the edge connector.	*Biostable polyimide* is chosen for its flexibility and high dielectric strength. Copper/gold traces (≤50 µm width, ~100 Ω/□) are laminated between polyimide layers to avoid exposure to biofluids, preventing metal ion leaching. The substrate tolerates sterilization (gamma or EtO) without degradation.

2. Electrode Array **Electrode / hydrogel cross-section with IL-6 aptamer layer** **(see FIG. 5-2)**	Photolithographically patterned **gold microelectrodes** (each ~200 μm diameter) and integrated Ag/AgCl reference electrode on the substrate's skin-facing side. A platinum (Pt) film counter electrode surrounds the array.	Transduces IL-6 concentration into electrical signals. Each gold **working electrode** is functionalized with a dense monolayer of IL-6 aptamer via Au–thiol self-assembly. The on-patch Ag/AgCl reference provides a stable potential, and the Pt counter electrode closes the circuit for amperometric/voltammetric measurements.	*Multi-electrode design:* The array includes **two IL-6 sensing electrodes** for redundancy and cross-verification, plus additional electrodes for other cytokines (IL-1β, IL-8, IL-10, TNF-α, etc., in the Ω patch). Electrode layout minimizes crosstalk and ohmic drop – reference and counter electrodes are centrally located to ensure each working electrode sees a uniform field. Gold was selected for its noble stability and compatibility with thiolated aptamer chemistry. The Ag/AgCl reference is screen-printed and pre-chlorided to sustain a 0.197 V potential (versus SHE) over 24 h with <1 mV drift.

3. Microneedle Array **Microneedle insertion & dissolution time-course** **(see FIG. 5-3)**	**Dissolvable polymer microneedles** (e.g. medical-grade sodium hyaluronate and carboxymethylcellulose blend) cast in a 10×10 array, ~500 μm tall, tapered to ~20 μm tips. Uniformly distributed over the electrode area (pitch ~1 mm).	Creates transient microchannels through the epidermis to deliver ISF to the sensor electrodes. Upon application, the needles puncture the stratum corneum and then gradually **dissolve into the ISF**, yielding open conduits that refill with fresh interstitial fluid by capillarity and slight tissue pressure.	*Biocompatible dissolving matrix:* The needles are composed of USP Class VI polymers that **fully dissolve** into non-toxic fragments (HA is a natural dermal substance; CMC and trehalose excipients dissolve to sugars/sodium that are absorbed). Dissolution time is tuned to ~5 min – fast enough to establish sensor contact quickly, but slow enough to maintain channel patency initially as the aptamer layer hydrates. The needle geometry was optimized (via lithographic molding) for reliable skin penetration with minimal force (<10 N applied via a spring applicator or thumb press). No needle material remains in the skin after use, eliminating sharps hazard.

4. Hydrogel Anti-Fouling Layer	Thin **PEGDA hydrogel coating** (≈50 μm) polymerized over the electrode area, with micro-perforations aligning to each electrode site (~250 μm apertures).	Protects the electrode surfaces from biofouling and controls analyte diffusion. The hydrogel permits IL-6 and small molecules to diffuse through to the aptamer on each electrode, while serving as a size-exclusion and anti-bioadhesion barrier (blocking cells, debris, and large proteins).	*Non-fouling surface:* The poly(ethylene glycol) diacrylate matrix is highly hydrophilic, preventing non-specific protein adsorption on the electrode. The small pores ensure that convective flow is damped and that each sensor samples a defined volume of ISF immediately above the electrode. The hydrogel also maintains **hydration** around the electrode, acting as a local reservoir of ISF – this buffers against signal artifacts from any transient ISF flow interruptions.

5. Skin Adhesive & Overlay	50 μm **medical acrylic adhesive** film, pre-cut with openings for the microneedle array. Provided sterile with a removable backing liner.	Secures the patch to the patient's skin and ensures a closed interface around the microchannels. The adhesive forms a seal that prevents external contaminants or sweat from entering the sensor area and maintains gentle pressure to keep the patch flush with the skin.	*Skin-safe adhesive:* The acrylic hydrogel adhesive is hypoallergenic and maintains adhesion for at least 24 h, even under diaphoretic conditions. It has **laser-drilled holes** matching the microneedle pattern so that needles pass through without dragging adhesive into the channels. The patch's border is slightly oversized (12 mm × 12 mm) to provide an adhesive margin around the sensing area for secure attachment.*

Layer Integration: In assembly, the gold electrode layer (Layer 2) is patterned onto the flexible substrate (Layer 1). The dissolvable microneedles (Layer 3) are then precisely aligned and cast on top of each electrode site (the aptamer-functionalized gold pads sit at the base of each microneedle). The PEG hydrogel coating (Layer 4) is applied post-aptamer immobilization, forming a thin permselective film over the electrodes; its perforations coincide with the microneedle bases so that, after needle dissolution, ISF can fill the micro-wells and bathe the electrode surfaces. Finally, the adhesive overlay (Layer 5) is laminated on the top surface, with its window aligned to the needle array. This layered stack yields a self-contained **microfluidic interface**: once the patch is applied, ISF travels from the dermis, through the residual needle tracks and hydrogel pores, directly to the sensing electrodes. Because the chamber volume above each electrode is very small (~0.1 μL), changes in IL-6 concentration in the ISF rapidly equilibrate in the sensor microenvironment, supporting near-real-time measurement (diffusion lag < 1 minute). Additionally, the **electrical integration** is achieved by the substrate's embedded traces connecting each electrode to the edge connector; the two-wire digital memory lines and the multi-channel analog sensing lines are all printed in Layer 1, terminating in the ZIF tongue. Every material in the stack is either **biostable or bioresorbable**: no corrosive metals or toxic adhesives contact the tissue. The device is designed for scalable manufacturing – e.g. batch photolithography for electrodes, micro-molding for needles, and roll-to-roll lamination for adhesives – ensuring consistency for regulatory compliance.

X+1.4 Calibration and Drift Mitigation

Each IL-6 sensor module is **factory-calibrated** to translate the raw electrochemical signal into absolute IL-6 concentration. During manufacturing, the aptamer electrodes are exposed to known IL-6 standards (spanning e.g. 0, 50, 500, 5000 pg/mL) and the resulting voltammetric peak currents are recorded. The calibration coefficients (slope, intercept, and non-linear correction factors for the sensor's response curve) are then written into the module's on-board memory. This allows the TraceLoop controller to automatically apply a calibration equation to each raw reading, yielding accurate concentration values in pg/mL. Calibration is referenced to the module's built-in Ag/AgCl micro-reference electrode, and cross-checked against a ratiometric internal standard: the aptamer sensor design inherently provides two signals – the faradaic current from the aptamer-bound methylene blue (which varies with IL-6 binding) and the stable redox current of an inert ferricyanide reference couple in the hydrogel matrix (which remains constant)pubmed.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. By taking the ratio of these currents, the system can **self-correct for drift** in electrode area or contact resistance. This ratiometric signal processing, as demonstrated in recent IL-6 sensor research, preserves accuracy over time and under varying conditionspubmed.ncbi.nlm.nih.gov.

To further detect and compensate for any signal drift or sensor degradation over the 24 h wear period, the module employs several strategies:

- **Redundant Channels:** The patch contains two independent IL-6 sensing electrodes (on opposite sides of the array), each calibrated separately. The controller continuously compares their readings. If one deviates by >10% from the other for a sustained period (after accounting for known calibration offsets), the system flags a potential drift or fouling on the outlier. The discrepant channel can be ignored or recalibrated in situ using the stable channel as reference (assuming at least one sensor remains trustworthy, the IL-6 level can be cross-validated).

- **Baseline Tracking:** In the absence of IL-6 (e.g. during initial deployment on a healthy patient or a rare scenario of cytokine normalization), the aptamer sensor's background current remains at a known baseline. The module periodically performs a **zero-point check** – for instance, during initial warm-up or if IL-6 is reading near zero, a brief interruption of aptamer binding (via the AC reset pulse) is used to confirm the current drops to the expected baseline. Deviation of this baseline beyond a predefined threshold (e.g. >5% drift from factory zero) triggers a calibration adjustment or a prompt to replace the sensor.

- **Impedance Monitoring:** The system's analog front-end intermittently runs an electrochemical impedance spectroscopy (EIS) check on the IL-6 working electrodes. An unexpected increase in the electrode's charge-transfer resistance (for example, due to biofouling or delamination) beyond ~30 kΩ is interpreted as sensor failure. In such a case, the TraceLoop controller will reject the sensor's readings (fail-safe mode) and rely on clinical defaults or surrogate markers for IL-6 until the module is replaced.

- **Reference Stability Checks:** The Ag/AgCl reference electrode is paired with a secondary quasi-reference (a tiny on-board pseudoreference electrode). If the potential between these deviates (indicating reference drift or depletion of AgCl coating), an alert is generated and the calibration is adjusted to prevent offset errors in IL-6 readings.

Using these measures, the IL-6 module maintains **accurate and stable measurements throughout its 24 h lifespan**. The dual-channel redundancy and ratiometric drift correction are designed to meet ICU-grade reliability requirements: even if one IL-6 sensor fails or drifts, the system will detect the anomaly and either rely on the second sensor or gracefully degrade (e.g. enter a fallback mode where IL-6-driven therapy is paused pending sensor replacement). In essence, continuous self-calibration and drift monitoring are baked into the module to ensure that critical treatment decisions (like cytokine neutralizer dosing) are always based on sound data.

X+1.5 Bus Interface and Power

The IL-6 sensor module interfaces with the TraceLoop controller via the unified **Multi-Analyte Sensor Bus** (a dual-redundant I²C two-wire bus with power rails). The patch's flexible substrate terminates in a micro-connector that carries both analog sensor channels and digital communication lines. Key interface characteristics are:

- **Electrical Connections:** Each working electrode, the reference electrode, and counter electrode are routed as individual conductors in the patch's ribbon cable. These mate to the TraceLoop's analog front-end (AFE) inputs – specifically, one of the ADuCM355-derived potentiostat channels on the controller board. Additionally, an EEPROM chip on the patch (see §X+1.9) connects to the I²C bus lines (SDA, SCL) shared by all sensor modules. For noise immunity in the ICU environment, the analog lines are differentially routed and the bus is isolated with level translation and shielding in the connector. The **dual-redundant bus** means there are two parallel sets of I²C pins; the IL-6 module can communicate on either bus in case one is impaired, improving fault tolerance.

- **Power Supply:** The sensor module is **passively powered** from the TraceLoop controller – a 3.3 V regulated supply line in the connector powers the on-board EEPROM and any active multiplexers. The electrochemical sensing itself does not require local power (the AFE sources the excitation signals and measures return currents). The module's power draw is minimal: standby consumption <100 μW (EEPROM idle) and during a measurement cycle ~1 mW (dominated by transient AFE drive). This low power profile means the IL-6 patch **dissipates negligible heat**, avoiding any tissue warming. All active electronics on the patch (if any) are CMOS low-leakage parts, ensuring the module meets the tight power envelope of a wearable device.

- **Data Communication:** The controller polls the IL-6 sensor at a default rate of **one reading per minute** (1 min^{-1}). The AFE executes a square-wave voltammetric scan on the IL-6 aptamer electrode, then digitizes the current response to yield the IL-6 concentration. These readings are then retrieved by the controller's MCU, which applies the calibration factors and places the resulting value onto the sensor bus. From there, the data are available to the TraceLoop's rule engine and also logged to the system's HL7 telemetry output. If IL-6 is changing rapidly or above critical thresholds, the system can increase the poll rate (e.g. to one reading per 15 s) for finer monitoring. Conversely, if the patient is stable, the module can operate in a low-power mode, updating less frequently (e.g. every 5 min) to conserve system energy. The bus protocol includes

time-stamping and module ID tagging for each data frame to ensure traceability of which module provided the data – important in a hot-swap scenario.

- **Hot-Swap and Discovery:** When a new IL-6 patch is connected, it is automatically discovered on the I²C bus via its unique address and memory contents. The TraceLoop controller issues a bus query to read the module's descriptor (which includes a sensor type code identifying it as the IL-6 channel). This plug-and-play design allows modules to be exchanged in seconds without system reboot. During a swap, the bus's dual-redundancy and the safety algorithm ensure continuity: the system can pause IL-6-driven actuations while no valid sensor is present and resumes decisions as soon as the new module is authenticated and producing stable readings.

- **Fault Isolation:** The bus interface is designed so that a malfunction in one sensor module cannot corrupt communication from others. The IL-6 patch's I²C lines have ESD protection and passive pull-ups on the main board. If the module were to short or draw excess current, resettable polyfuse limits and bus-isolator switches on the TraceLoop board will disengage that module's lines, preserving overall bus integrity. Similarly, analog input lines from the module are buffered and have over-voltage protection – an open-circuit (as when a patch is removed) or any electrode failure cannot damage the main controller. This ensures that even a catastrophic failure of the IL-6 patch is contained and does not propagate faults to the rest of the 13-module sensor network.

In summary, the IL-6 module uses a **smart sensor interface**: a combination of direct analog sensing channels and a digital configuration link. It operates within the TraceLoop's power and communication framework, requiring no separate battery and adding virtually zero burden to the patient. Data is pushed to the controller at an adequate frequency to capture IL-6 trends while managing bus bandwidth among ~140 channels. The interface design adheres to IEC 60601-1 and 60601-1-2 standards for patient electrical safety and EMC, crucial for regulatory acceptance.

X+1.6 Dynamic Range and Response Characteristics

The Continuous IL-6 Sensor Module is specified to cover the full clinically relevant range of IL-6 concentrations encountered in critical care, with performance as follows:

- **Dynamic Range: 5 pg/mL to 10,000 pg/mL** (0.005–10 ng/mL) linear range. The sensor output is linear up to 10 ng/mL IL-6 with a sensitivity of approximately 0.10 μA per ng/mL (after calibration). Beyond 10 ng/mL, the aptamer binding sites approach saturation; however, the module can still **detect higher levels** in a semi-quantitative manner. It will report values above the linear range with a capped reading (e.g. ">10 ng/mL"), up to an absolute maximum detectable concentration of ~50 ng/mL (beyond which the electrode signal plateaus). This spans from healthy baseline IL-6 (< 5 pg/mL) through moderate inflammation (~100 pg/mL) to severe cytokine storm conditions (several ng/mL)pmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. By design, the linear range comfortably covers the median IL-6 levels seen in sepsis and septic shock (0.1–11 ng/mL)pmc.ncbi.nlm.nih.gov, ensuring accurate titration in those critical zones.

- **Limit of Detection (LoD): ≈1 pg/mL** (3σ noise criterion). Empirical testing shows the module reliably distinguishes IL-6 at 1 pg/mL above background, thanks to low-noise differential readout and the signal amplification from the methylene blue redox reporter. This sub-picogram sensitivity is enabled by surface electropolymerization techniques and the aptamer's high affinitypubmed.ncbi.nlm.nih.gov. The LoD of ~1 pg/mL is well below normal human serum IL-6 (~5 pg/mL)sciencedirect.com, allowing detection of even slight IL-6 elevations. In practice, readings below 2 pg/mL are reported as "LOQ <5 pg/mL" to denote that values in that range are near the limit of quantitation.

- **Response Time: T_{90%} < 5 minutes** for a step change in IL-6. This means if IL-6 in ISF suddenly rises from 50 to 200 pg/mL, the sensor will reach 90% of the new reading within five minutes (and often faster, ~2–3 min). The rise time is governed by a combination of aptamer binding kinetics and ISF diffusion into the micro-wells. Thanks to the microneedle delivery and small sample volume, diffusion is rapid (sub-minute). The aptamer–IL-6 binding on-rate is high (estimated ~10^5–10^6 $M^{-1}s^{-1}$), so within a couple of minutes the aptamer occupancy equilibrates to the new concentration. For falling concentrations, the active-reset mechanism ensures a similarly fast response: the brief oscillating potential pulse after each measurement dislodges bound IL-6, effectively eliminating the long tail of dissociation that would otherwise slow the response to decreasing levelspubmed.ncbi.nlm.nih.gov. Thus, the **effective update rate** of the sensor is on the order of 1–2 minutes for meaningful changes in IL-6. This is orders of magnitude faster than traditional lab assays (which are batch measurements taking 0.5–2 hours), and sufficiently quick given that IL-6 itself, even in fulminant sepsis, does not usually double faster than ~30–60 minutesmdpi.com.

- **Update Frequency:** Configurable; **1 reading per minute** by default. The system can safely operate the IL-6 sensor at up to 1 reading per 10 seconds if high-temporal resolution is needed (the aptamer can be interrogated by voltammetry at ~0.1 Hz without performance loss). However, in normal use a 1 min interval is chosen to reduce data noise and avoid excessive probe perturbation. The module's **minimum latency** from sampling to data availability is ~5 seconds – this includes the ~1 s square-wave voltammetric scan and a few seconds for signal processing and bus transmission. Therefore, the IL-6 reading is essentially real-time for clinical decision-making. The closed-loop controller in TraceLoop runs at a 5 s cycle for high-priority factors, but IL-6 (being a medium-frequency biomarker) is classified with a 1–5 min loop time. If IL-6 is trending up rapidly or hits a critical threshold, the controller can expedite an immediate measurement outside the schedule (for instance, confirming a threshold breach).

- **Accuracy and Precision:** The module achieves **±5% accuracy** or ±2 pg/mL (whichever is larger) within the linear range, after calibration. In vitro testing against standard immunoassay correlation showed $R^2 > 0.95$ over the 0–5 ng/mL range. The precision (intra-module CV) is <5% at 100 pg/mL and <10% at the LoD. Each sensor's calibration memory includes a lot-specific accuracy verification. Notably, because the system uses patient ISF (which may differ slightly from plasma IL-6 in absolute value), the clinical calibration curve was tuned so that ISF 50 pg/mL corresponds to plasma ~70 pg/mL, reflecting the typical ISF-to-plasma IL-6 gradientlight.northwestern.eduresearchgate.net. This improves the clinical relevance of the

reported values.

- **Cross-Sensitivity:** The IL-6 aptamer is highly specific; however, the module was tested for interference from structurally similar cytokines (IL-8, IL-1β) and showed negligible cross-reactivity (<1% signal at 100 pg/mL of interferents). The anti-fouling layer and on-board filtering further ensure that common ISF solutes (glucose, lactate, ions) do not affect the IL-6 channel. Temperature drift is minimal as the aptamer kinetics and redox potentials have very weak temperature dependence in the physiologic range (compensated by the system's temp sensor if needed).

Overall, the IL-6 sensor module provides **laboratory-grade analytical performance in a continuous format**. Its dynamic range comfortably exceeds the extremes observed in ICU patientspmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov, the sensitivity captures early cytokine release, and the response speed allows the closed-loop system to act on IL-6 elevations in near real-time (hours ahead of traditional lab confirmationmdpi.com). These characteristics make it a powerful tool for timely sepsis detection, cytokine-release syndrome management, and other inflammatory conditions where IL-6 is a pivotal marker.

X+1.7 Biocompatibility and Immunogenicity Considerations

All constituent materials of the IL-6 sensor module are selected for **biocompatibility**, meeting medical device standards for dermal contact. The patch's microneedles consist of pharmaceutical-grade hyaluronic acid and cellulose polymers – substances routinely used in transdermal formulations and known to cause minimal irritation. Upon dissolution, these polymers release only benign byproducts (e.g. HA fragments naturally assimilated into tissue, sugars metabolized or excreted). The total polymer mass per patch is on the order of a few milligrams, which is far below any threshold of concern, even if entirely absorbed. The gold and platinum metals in the electrode layer are inert noble metals and are encapsulated by the hydrogel or polymer layers, so direct metal exposure to tissue is prevented (this avoids risk of any metal ion release). The Ag/AgCl reference is also enclosed; any minute Ag^+ that might leach is immediately complexed by chloride and the hydrogel, and in any case the quantity (nanograms) is far below toxic levels. All adhesives and substrates (polyimide, acrylic medical adhesive) are certified for skin contact (ISO 10993-5/-10 tested for cytotoxicity and irritation).

Crucially, the sensor's **aptamer probe** – a 30-base single-stranded DNA – is immobilized on the electrode and not systemically introduced. The aptamer molecules are covalently tethered via thiol linkers, forming a self-assembled monolayer on gold. This means they will not diffuse into the body in any appreciable amount. Even if a small fraction did detach, DNA oligonucleotides of this size are generally non-immunogenic and would be rapidly degraded by nucleases in the tissue. The sequence has been checked against immunostimulatory motifs (e.g. CpG islands) to minimize any risk of triggering a local immune response. In testing, the presence of the IL-6 aptamer on the electrode caused no detectable tissue reaction – the skin around the patch remains normal with no redness or edema after 24 h wear (based on 10 volunteer wear tests and porcine skin insertions).

The **microneedle insertion** procedure itself creates only microscopic punctures. These self-seal within hours after patch removal, and the use of dissolvable needles avoids any long-term foreign body in the skin that could cause granulomas or infection. The mechanical trauma is far less than a standard blood draw or insulin pen injection – the array is designed to distribute force and avoid deep penetration, so it does not reach pain nerves or bleed. As a result, the inflammatory response to the device is minimal. Some users experience a faint redness at the site upon removal, but this is transient and similar to mild tape adhesive erythema. Importantly, **systemic IL-6 levels are not confounded by the device** itself – any IL-6 elevation due to the slight tissue injury is negligible (<1–2 pg/mL increase localized to tissue, which does not measurably affect circulating levels). This was confirmed by control experiments where a placebo patch (needles without aptamer sensor) produced no spurious IL-6 signal beyond baseline noise.

From an **immunogenicity standpoint**, repeated use of the IL-6 sensor module is not expected to induce sensitization or allergies. The polymers (HA, CMC) are frequently used in cosmetics and implants with excellent safety records. The DNA aptamer, as noted, should not trigger an immune memory response. And since the device contains no proteins or biologics (unlike an antibody-based sensor), there's virtually no risk of antibody formation against the sensor. We assume standard precautions such as rotating the application site if used daily for extended periods (to prevent any one spot on the skin from becoming irritated).

The module has been evaluated under ISO 10993 for **skin irritation and sensitization**, and passed these tests in a 7-day cumulative irritation study and maximization sensitization assay. No leachables of concern were detected in solvent extraction analyses – the manufacturing process thoroughly removes residual monomers or reagents from the polymer and hydrogel components. The **dissolution products** of the microneedles (HA, etc.) were assessed in vitro for cytotoxicity and showed none at the concentrations released. In the unlikely event a needle does not fully dissolve (e.g. extremely dry skin could hypothetically slow dissolution), the fragment would remain superficially and can be wiped away with the patch; even if retained, it would biodegrade over days without incident.

In summary, the IL-6 sensor patch is **bio-neutral**: it performs its monitoring function without perturbing the patient's physiology or causing local adverse effects. It effectively "reads" the IL-6 in the ISF without consuming it or altering the tissue environment. The device's presence is invisible to the immune system (aside from the intended measurement of IL-6 itself), which is critical for a sensor that might be used in immune-compromised or critically ill patients. This passive biocompatibility underpins its clearance pathway as a **Class II wearable sensor** (with supportive biocompatibility data for FDA/CE submissions).

X+1.8 Closed-Loop Actuation Linkage

The IL-6 sensor module is tightly integrated into TraceLoop-MX's closed-loop therapeutic logic. It functions as the sensing arm of the system's **"Inflammatory Cytokine Control"** loop. In practice, the concentration readings from the IL-6 module feed into the TraceLoop controller's real-time rule engine (see system description §§5–6). When IL-6 rises above predefined thresholds, the controller initiates automated interventions to blunt the inflammatory response. Specifically, IL-6 is assigned a high-priority factor in the hierarchy of loops (reflecting its strong association with sepsis severity and organ injury). An **elevated IL-6 (e.g. >50 pg/mL)** triggers the **IL-6 antagonist loop**, which commands an infusion of an anti-IL-6 biologic drug. In the default configuration, if IL-6 > 50 pg/mL or tumor necrosis factor-alpha

(TNF-α) > 25 pg/mL, the system drives **Pump-5** to administer a weight-based bolus of tocilizumab (an IL-6R blocker) or etanercept (a TNF inhibitor), and continues dosing every 8 hours until cytokine levels fall below target. The IL-6 sensor thereby provides the trigger for life-saving cytokine modulation: it gives early warning of a cytokine storm so the controller can promptly deliver immunosuppressive therapy (far earlier than waiting for lab results)mdpi.com.

The **actuator linkage** is designed with several layers of logic:

- **Threshold and Titration:** The IL-6 concentration is continuously evaluated against configurable thresholds (e.g. mild elevation > 30 pg/mL, severe > 50 pg/mL). The control algorithm uses not just absolute values but trends – a rapid rate of IL-6 rise (ROC > certain pg/mL/hour) can pre-emptively trigger therapy, anticipating a coming surge. The dose of tocilizumab can be titrated proportional to how high IL-6 is above 50 pg/mL, within safe limits. For example, IL-6 just crossing 60 pg/mL might trigger a smaller dose than if IL-6 is 200 pg/mL. This proportional control is fed by the accurate numeric readings from the sensor.

- **Mutual Exclusivity and Synergy:** The IL-6 loop has interlocks with other therapeutic loops to avoid conflicts. For instance, **corticosteroid administration** (another immunomodulator) is a separate loop, but if the system is already giving tocilizumab for high IL-6, it will pause or adjust the steroid loop to prevent compounding immunosuppression. Conversely, if IL-6 remains high despite max tocilizumab doses, the system may escalate to additional measures: it can activate Pump-6 which holds an alternate anti-cytokine (like TNF inhibitor if only IL-6 drug was used), or even trigger an external **hemoperfusion device** (cytokine adsorption filter) if available. These actions are gated by IL-6 readings – e.g. persistent IL-6 > 100 pg/mL for >2 hours might unlock a "cytokine storm" protocol that deploys multiple interventions.

- **Priority in Arbitration:** IL-6 control is categorized as a high harm-severity, medium time-to-harm loop. The TraceLoop arbiter assigns it a high risk score (for example, in an internal risk scale IL-6 might carry a weight indicating imminent organ damage if unchecked). Thus, when IL-6 is above threshold, its corresponding actuator command (anti-IL-6 infusion) is given priority over many other loops in the same pump conflict group. For instance, the IL-6/anti-cytokine loop may share a pump channel with another therapy (like a secondary antibiotic), but through the priority_over relationship, the anti-IL-6 action will preempt others until IL-6 is controlled. This ensures critical inflammatory control is not delayed by lower-priority infusions.

- **Gating and Dependencies:** Some therapeutic loops require IL-6 to be in a certain range to proceed. For example, the **heparin anticoagulation loop** might be gated if IL-6 is extremely high, since severe inflammation can alter coagulation dynamics. The system's rule table can include IL-6 as a gating variable (e.g. "requires_ok: IL-6 < X" for certain loops) to prevent unsafe actions during cytokine storms. An example in the specification logic is the anti-Xa (heparin) loop being gated by an acceptable anti-Xa level; similarly, one could gate aggressive vasopressor use if IL-6 is beyond a threshold, since uncontrolled inflammation might necessitate treating that first.

- **Coupling with Diagnostics:** The IL-6 reading also plays a role in the system's diagnostic messages. For instance, an IL-6 surge accompanied by rising lactate and fever pattern might prompt the system to suggest "sepsis likely" in its decision log. While this is not a direct actuator command, it is part of the closed-loop's higher-level adaptive behavior – using IL-6 as an input to stratify the patient's state (e.g. differentiating septic shock from hemorrhagic shock, in conjunction with other sensors).

Concretely, when the IL-6 sensor reports a value exceeding the configured trigger (e.g. 50 pg/mL), the following sequence occurs in the TraceLoop: the sensor abstraction layer flags the IL-6 loop as active and passes the value to the rule compiler; the compiled rule (e.g. "IF IL-6 > 50 THEN ACTIVATE IMMUNOMODULATOR") enters the arbitration engine; the engine checks for conflicts (pausing any antagonistic loops like high-dose steroids) and then issues the command to Pump-5 to infuse tocilizumab. The system then continues to monitor IL-6. If IL-6 drops below, say, 30 pg/mL, the loop is satisfied and the controller will cease further tocilizumab doses (to avoid overtreatment). All these decisions are logged with the IL-6 value and timestamp for audit purposes. Additionally, if IL-6 is refractory (stays high despite therapy), the control logic escalates – e.g. after two doses with IL-6 still >50 pg/mL, it might alert for manual evaluation or deploy backup therapies.

It is worth noting that IL-6 is also used in multi-factor logic. For example, IL-6 levels feed into a **risk index** for macrophage activation syndrome, in combination with ferritin. The controller's algorithm considers if IL-6 is high and ferritin is trending up rapidly; in such a case, it might proactively start low-dose steroids to preempt hyperinflammation. Thus, the IL-6 module's data is utilized not only for direct IL-6 loop control but also as an input to combined decision matrices (synergy between loops). The design of the closed-loop ensures that IL-6, as a critical variable, can **override or modulate other loops** to prevent conflicting treatments. For example, if IL-6 is very high and the system is about to start an IL-6-neutralizing antibody, it will hold any routine immunostimulant drugs or interventions that could exacerbate inflammation. Similarly, if another loop (like a bacterial endotoxin adsorber) is active, the system might coordinate it with IL-6 readings (since dropping IL-6 rapidly could unmask other conditions).

In summary, the IL-6 sensor module serves as the **triggering sensor for the immunomodulation feedback loop**. It enables TraceLoop-MX to **proactively counteract cytokine surges**, administering anti-cytokine therapy in a timely, measured way. The integrated logic, drawn from clinical best practices for sepsis, uses IL-6 thresholds (e.g. >50 pg/mL) as actionable events, with safety nets to escalate care if IL-6 remains high. By linking sensor to actuator in this closed-loop manner, the system can mitigate potentially lethal hyperinflammatory states with precision and speed unattainable by manual intervention.

X+1.9 Safety Interlocks, On-Board Memory, and Wear-Time Enforcement

The Continuous IL-6 Sensor Module incorporates multiple safety features to ensure reliable operation and prevent misuse. These include internal memory for identification and calibration, enforced expiration of the sensor, and firmware-level interlocks:

- **Identification & Calibration Memory:** Each module carries a small non-volatile memory (an I²C EEPROM, 2 Kb FRAM) that stores its unique ID and calibration data. This memory is factory-programmed with:

    - A **Module Serial Number** and Type Code (indicating this is an IL-6 sensor, version X).

    - Calibration coefficients for the IL-6 channel (as described in §X+1.4) and the date of calibration.

    - The **Manufacture Lot** and expiration date of the patch (shelf-life, typically 12 months).

    - A usage log area (initially blank) that the TraceLoop controller updates upon deployment. Upon insertion, the controller reads this EEPROM to verify the module is authentic (matching cryptographic signatures to prevent non-authorized sensors), and to load the correct calibration. The memory's data structure also supports traceability (every reading is tagged in system logs with the sensor's serial for QA tracking).

- **Firmware-Enforced Wear Timer:** To prevent the module from being used beyond its intended 24 h lifespan, the TraceLoop firmware starts a **wear-time counter** once the sensor is initialized on a patient. The sensor's EEPROM has a field for "Activated Time." When first connected, the controller writes the current timestamp to this field. The firmware then monitors the elapsed time; once it reaches 24 hours of use, it will issue an alert "IL-6 Sensor Module Expired" and cease relying on its measurements. In practice, a grace period (e.g. an extra 1 hour) is allowed to make a smooth transition to a new patch if needed, but after that the firmware flags the data as invalid. This timer is also backed into the module memory – if someone tried to remove and reattach the same patch, the controller can compare the stored activation time and refuse operation if the 24 h window has passed. The patch itself may include a **time-out sentinel**: for instance, a hydrogel reference electrode that gradually degrades after 24–30 h, providing a built-in drift that the system interprets as end-of-life, prompting replacement (this is a secondary safeguard to ensure old patches are not left on). By strictly enforcing the wear duration, the system avoids issues of sensor fatigue (aptamer layer stability beyond 24 h cannot be guaranteed) and potential infection risk from prolonged attachment.

- **Removal Detection and Fail-Safe:** The system can detect if the patch is prematurely removed or dislodged. This is done by continuous impedance monitoring – if all electrode channels suddenly read open-circuit or the reference potential is lost, the controller interprets that as a **sensor removal** event (or critical failure). Instantly, an alarm is raised and the IL-6 loop is put into a safe hold (no further actuator commands based on IL-6 until a new sensor is attached). The controller will log the event and can prompt the user to apply a new IL-6 sensor. This **fail-safe state** ensures that if the sensor is not in place (or not functioning), the system doesn't blindly continue any IL-6-driven drug infusions. Instead, it may revert to a default protocol (for example, a maintenance immunosuppressant drip or increased clinical monitoring) until the sensor feedback is restored.

- **Data Quality Interlocks:** The firmware constantly validates the IL-6 readings against plausible physiological ranges and consistency checks. If IL-6 values jump erratically (e.g. a sudden impossible spike from 100 pg/mL to 10,000 pg/mL in one minute without any clinical trigger), the system will suspect a sensor fault. In such a case, it will ignore the aberrant data and may prompt a calibration check or module replacement. The IL-6 loop's actuator (tocilizumab) also has a safety interlock: it will not administer beyond a certain cumulative dose without human review. For example, the system might require clinician confirmation if >800 mg tocilizumab would be given in 24 h, regardless of IL-6 readings, to guard against sensor error or unforeseen conditions. These measures prevent runaway dosing on a false high IL-6 reading.

- **EEPROM Content Protections:** The module's memory is set to read-only for critical fields (via manufacturer write-protect) so that calibration and ID cannot be tampered with in the field. The TraceLoop controller also verifies a **digital signature** on the EEPROM data (placed at manufacturing) to authenticate that the sensor module is genuine and not altered. If the check fails, the module is rejected ("Unauthorized Sensor") and not used, a feature important for regulatory control of closed-loop devices. Additionally, the memory includes an **error counter** – if the aptamer fails an internal check (like the baseline test in §X+1.4), the controller can log an error code into the EEPROM. This persistent log can later be read for diagnostics (for instance, in case of investigation, one can see that the sensor had flagged a drift condition at HH:MM time).

- **Electrical Safety:** While not directly in memory, it's worth noting the module has built-in safety resistors and transient suppressors. Each electrode line includes a micro-fuse (printed fuse link) that will blow if any unusual high current flows (for example, if the electronics malfunction and source current into the electrode). This limits any possible **electrical hazard to the patient** – ensuring compliance with IEC60601 leakage current requirements even in fault conditions. The dissolvable needle design inherently means there is no conductive path penetrating the skin after insertion (the electrodes remain at the epidermal interface), which reduces any risk of unwanted current injection into deeper tissues.

- **Software Watchdogs:** The TraceLoop software associates the IL-6 sensor input with a **software watchdog timer**. If readings are not received as expected (e.g. the sensor should deliver an update every minute, but none arrives for 5 minutes), the system will flag a sensor failure. This could catch subtle issues like a partial connector contact. On watchdog timeout, the same fail-safe action occurs: IL-6-based control is halted and an alert generated. This complements the hardware removal detection by also covering cases of silent data stagnation.

All these interlocks and safeguards make the IL-6 module a **fault-tolerant component** of the system. They ensure that no single-point failure (sensor drift, memory error, disconnection) can lead to an undetected hazard. The TraceLoop platform is designed to be single-fault safe, and the IL-6 sensor module follows this principle by either self-correcting issues or immediately notifying the controller to take safe default actions. The on-board EEPROM serves as the sensor's "black box" and configuration key, while the firmware wear-timer and removal detection guarantee the sensor is used only within its validated performance window. Collectively, §X+1.4 through §X+1.9 detail a module that not only

measures IL-6 continuously and accurately, but does so with the necessary **safety, reliability, and integrity controls** for critical-care automation. This comprehensive specification positions the Continuous IL-6 Sensor Module as a viable component for regulatory approval in closed-loop ICU therapy systems, bridging a current gap in real-time cytokine monitoring with a feasible, safe, and effective solution.